

Vol. X, Part II

April, 1940

THE
INDIAN JOURNAL
OF
AGRICULTURAL SCIENCE

Issued under the authority
of
The Imperial Council of Agricultural Research



Annual subscription
Rs. 15 or 23s. 6d.

Price per part
Rs. 3 or 5s.

PUBLISHED BY THE MANAGER OF PUBLICATIONS, DELHI.
PRINTED BY THE MANAGER, GOVERNMENT OF INDIA PRESS, NEW DELHI.
1940

List of Agents in India from whom Government of India Publications are available.

(a) PROVINCIAL GOVERNMENT BOOK DEPOTS.

- ASSAM** :—Superintendent, Assam Secretariat Press, Shillong.
BIHAR :—Superintendent, Government Printing, P. O. Gulzarbagh, Patna.
BOMBAY :—Superintendent, Government Printing and Stationery, Queen's Road, Bombay.
CENTRAL PROVINCES :—Superintendent, Government Printing, Central Provinces, Nagpur.
MADRAS :—Superintendent, Government Press, Mount Road, Madras.
NORTH-WEST FRONTIER PROVINCE :—Manager, Government Printing and Stationery, Peshawar.
ORISSA :—Press Officer, Secretariat, Cuttack.
PUNJAB :—Superintendent, Government Printing, Punjab, Lahore.
SIND :—Manager, Sind Government Book Depot and Record Office, Karachi (Sadar).
UNITED PROVINCES :—Superintendent, Printing and Stationery, U. P., Allahabad.

(b) PRIVATE BOOK-SELLERS.

- Advani & Co., The Mall, Cawnpore.
 Aero Stores, Karachi.
 Army Musketry Stores, Moga (Punjab).
 Banthiya & Co., Ltd., Station Road, Ajmer.
 Bengal Flying Club, Dum Dum Cantt.
 Bhawnani & Sons, New Delhi.
 Bombay Book Depot, Charni Road, Girgaon, Bombay.
 Book Company, Calcutta.
 Booklover's Resort, Taikad, Trivandrum, South India.
 British Book Depot, Lucknow.
 British Book Depot, Rialpore.
 British Stationery Mart, Booksellers, Peshawar Cantt.
 Buckingham & Co., Booksellers and Stationers, Greenwood Street, Slakot City.
 Burma Book Club, Ltd., Rangoon.
 Cambridge Book Co., Booksellers, New Dak Bungalow Road, Patna.
 Chandrakant Chimanlal Vora, Ahmedabad.
 Chatterjee & Co., 3, Bacharam Chatterjee Lane, Calcutta.
 Chitney & Sons, Booksellers, etc., Dhantoli, Nagpur, C. P.
 Chukerverty Chatterjee & Co., Ltd., 13, College Square, Calcutta.
 Das Gupta & Co., 54/3, College Street, Calcutta.
 Dastane Brothers, Home Service, 456, Raviwar Peth, Poona 2.
 Delhi and U. P. Flying Club, Ltd., Delhi.
 Deshmukh Book Depot, Station Road, Sholapur.
 English Book Depot, Ferozepore.
 English Bookstall, Karachi.
 English Book Depot, Taj Road, Agra.
 English Book Store, Abbottabad, N.-W. F. P.
 Faqir Chand Marwah, Peshawar Cantonment.
 Higginbothams, Madras.
 Hindu Library, 137-F, Balaram De Street, Calcutta.
 H. L. College of Commerce, Co-operative Stores, Ltd., Ahmedabad.
 Hyderabad Book Depot, Chaderghat, Hyderabad (Deccan).
 Ideal Book Depot, Rajpur Road, Dehra Dun and Bombay Bazar, Meerut.
 Imperial Book Depot & Press, Near Jama Masjid (Machliwalan), Delhi.
 Imperial Publishing Coy., Lahore.
 Indian Army Book Depot, Dayalbagh, Agra.
 Indian Army Book Depot, Daryaganj, Delhi.
 Indian School Supply Depot, Central Avenue, South, P. O. Dharamtala, Calcutta.
 International Book Service, Poona 4.
 Jain & Bros., Sarafa Road, Gwalior, Messrs. M. B. Jain & Bros., Mori Gate, Delhi, and Connaught Place, New Delhi, Messrs. J. M.
 Joshi News Agent, Deygad Baria, via Piprod, Bombay, Mr. V. G.
 Kamala Book Depot, 15, College Square, Calcutta.
 Kansil & Co., 9, Commercial Buildings, The Mall, Lahore, Messrs. N. C.
 Karnataka Sahitya Mandir, Publishers and Direct Importers, Dharwar, (S. Sind).
 Keale & Co., 65, Britto Road, Karachi (Saddar).
 Kitabistan 17-A, City Road, Allahabad.
 Krishnaswami & Co., Teppakulam P. O., Trichinopoly Fort, Messrs. S.
 Lahiri & Co., Ltd., Calcutta, Messrs. S. K.
 London Book Co. (India), Arbab Road, Peshawar, Murree, Nowshera, Rawalpindi.
 Lyall Book Depot, Lyallpore.
 Malhotra & Co., Post Box No. 94, Lahore, Messrs. U. P. Mathur & Co., Messrs. B. S., Chatur-Vilas, Paota, Civil Lines, Jodhpur (Rajputana).
 Minerva Book Shop, Anarkali Street, Lahore.
 Modern Book Depot, Bazar Road, Slakot Cantt.
 Mohaulal Dossabhai Shah, Rajkot.
 Mohan News Agency, Booksellers, etc., Kotah (Rajputana).
 National Welfare Publicity Ltd., Mangalore.
 New Book Co., "Kitab Mahal", 188-90 Hornby Road, Bombay.
 Newman & Co., Ltd., Calcutta, Messrs. W. Oxford Book and Stationery Company, Delhi, Lahore, Simla, Meerut and Calcutta.
 Parikh & Co., Baroda, Messrs. B.
 Pioneer Book Supply Co., 20, Shib Narayan Das Lane, Calcutta and 219, Cloth Market, Delhi.
 Popular Book Depot, Grant Road, Bombay.
 Punjab Religious Book Society, Lahore.
 Punjab Sanskrit Book Depot, Lahore.
 Raghunath Prasad & Sons, Patna City.
 Rama Krishna & Sons, Booksellers, Anarkali, Lahore.
 Ram Krishna Bros., Opposite Bishrambag, Poona City.
 Ramesh Book Depot and Stationery Mart, Kashmir Gate, Delhi.
 Ray & Sons, 43, K. & L. Edwardes Road, Rawalpindi, Murree and Peshawar, Messrs. J.
 Reliance Stores, Booksellers, Princess Street, Karachi.
 Roy Chowdhury & Co., 72, Harrison Road, Calcutta, Messrs. N. M.
 Saraswati Book Depot, 15, Lady Hardinge Road, New Delhi.
 Sarcar & Sons, 15, College Square, Calcutta, Messrs. M. C. Sharada Mandir, Ltd., Nai Sarak, Delhi.
 Standard Book Depot, Cawnpore.
 Standard Book Depot, Lahore, Delhi and Simla.
 Standard Bookstall, Karachi.
 Standard Bookstall, Quetta.
 Standard Law Book Society, 79/1, Harrison Road, Calcutta.
 Subhan Bookseller & Publisher, Bilaspur, C. P., Mr. M. A.
 Swaminatha Shivam & Co., Paddukotah, Messrs. P. N. Tanawada & Sons Booksellers, Sangli.
 Tara & Sons, Razmak (India), Messrs. B. S. Taraporevala Sons & Co., Bombay, Messrs. D. B. Thacker & Co., Ltd., Bombay.
 Thacker, Spink & Co. (1933), Ltd., Calcutta.
 Tripathi & Co., Booksellers, Princes Street, Kalbadevi Road, Bombay, Messrs. N. M.
 Universal Books Depot, Booksellers, News Agents, etc., Kakul.
 University Book Agency, Kacheri Road, Lahore.
 Upper India Publishing House, Ltd., Literature Palace, Ammuddaula Park, Lucknow.
 Varadachary & Co., Madras, Messrs. P.
 Venkatasubban, A., Law Bookseller, Vellore.
 Wheeler & Co., Allahabad, Calcutta and Bombay, Messrs. A. H.
 Young Man & Co. (Regd.), Egerton Road, Delhi.

* Agent for Army publications only.

† Agents for publications on Aviation only.

ORIGINAL ARTICLES

FINAL REPORT ON THE SCHEME OF INVESTIGATION ON THE WHITE-FLY OF COTTON IN THE PUNJAB

BY

M. AFZAL HUSAIN, M.A. (CANTAB), I.A.S.
Vice-Chancellor, University of the Punjab, Lahore

AND

K. N. TREHAN
Punjab Agricultural College, Lyallpur

(Received for publication on 25 September 1939)

GENESIS OF THE SCHEME

THE white-fly of cotton was first noticed in the Punjab in 1915. In 1922 it was reported as a serious pest of cotton in the Montgomery district, and casual observations were started by the Entomological Section of the Department of Agriculture, Punjab. By 1925 the insect had increased so greatly that it called for special attention. Finally in 1928 a special assistant was deputed for this investigation. In April, 1929, the Indian Central Cotton Committee sanctioned a senior scholarship for this work and appointed Mr. Kidar Nath Trehan for two years.

A three years scheme of investigation was sanctioned in 1931 at a total expenditure of Rs. 28,132. The scheme was further extended for two years and the total expenditure for these two years was Rs. 17,574. A further extension was given for writing up the results at a cost of Rs. 3,029.

A supplementary scheme for spraying trials was sanctioned for one year (1933-34) at a cost of Rs. 11,250. In the following year (1934-35) spraying trials scheme was sanctioned at a cost of Rs. 4,365. The Indian Central Cotton Committee have also sanctioned a sum of Rs. 4,100 to undertake sprayings during a bad white-fly year.

PUBLICATIONS

The results of the investigations carried out have been published in the following contributions :—

1. Afzal Husain, M., 1930.—‘A Preliminary Note on the White-fly of Cotton in the Punjab’ :—*The Agricultural Journal of India*, Vol. XXV, Part VI, pp. 506-25
2. Afzal Husain, M. and Trehan, K. N., 1933.—‘Observations on the Life-history, Bionomics and Control of the White-fly of Cotton (*Bemisia gossypiperda* M. and L.)’ :—*Indian Journal of Agricultural Science*, Vol. III, Part V, pp. 701-53

3. Afzal Husain, M., Trehan, K. N. and Verma, P.M., 1936.—‘ Studies on *Bemisia gossypiperda* M. and L. No. 3, Seasonal Activities of *Bemisia gossypiperda* M. and L. (the White-fly of Cotton) in the Punjab ’:—*The Indian Journal of Agricultural Science*, Vol. VI, Part IV, pp. 893-903
4. Afzal Husain, M., Puri, A. N., and Trehan, K. N., 1936.—‘ Cell Sap Acidity and the Incidence of White-fly (*Bemisia gossypiperda* M. and L.) on Cottons ’:—*Current Science*, Vol. IV, Part VII, pp. 486-7.
5. Afzal Husain, M., Trehan, K. N., and Verma, P. M., 1939.—‘ Economics of Field Scale Spraying against the White-fly of Cotton (*Bemisia gossypiperda*, M. and L.) in the Punjab ’:—*The Indian Journal of Agricultural Science*, Vol. IX, Part I, pp. 109-26.

The following papers have recently been submitted for publication :—

1. Nature and Extent of Damage caused by White-fly of Cotton.
2. Further Observations on the Bionomics of the White-fly of Cotton.

In view of the fact that a great deal of material obtained has already been printed and the rest is ready for publication, only a brief summary giving the main results is being presented.

BRIEF SUMMARY OF THE RESULTS OF INVESTIGATION

During the five years that the scheme has run, a thorough study of the external characters, life-history and bionomics of the white-fly of cotton has been made, its seasonal activities have been studied and the nature and extent of damage done by it to the cotton crop has been assessed. In addition insecticidal method of control has been worked out on a field scale. Incidentally our work has also established that the white-fly is not the main factor in causing ‘ cotton failure ’ in the Punjab.

Distribution

An intensive study of the distribution of *Bemisia gossypiperda* has been carried out. This insect is widely distributed in the Punjab and has been recorded, from cottons or other alternative food plants, from almost all the districts of the province up to a height of 4,900 ft. above sea level. The severest of attack is, however, confined to Lyallpur, Jhang, Shahpur (Sargodha), Multan and Montgomery districts, the so-called ‘ canal colony tracts ’. This white-fly probably occurs in other parts of India also but exact information on this point is lacking. In Sudan and certain parts of Africa it is known chiefly as a vector of the ‘ leaf-crinkle ’ disease of cotton and tobacco.

Description of various stages

Egg.—Almost oval, stalked, light yellow when freshly laid, subsequently changing to dark brown.

Nymph of 1st instar.—Oval, light yellow, margin of body with 16 pairs of bristles. Legs functional.

Nymph of 2nd instar.—Oval, depressed, pale greenish yellow, legs degenerate, without any joints.

Nymph of 3rd instar.—Shape and colour identical with nymph of 2nd instar.

Pupa.—Body slightly convex, deep yellow. Spines on the back variable in number.

Adult.—Body yellow with two pairs of white wings. Eyes constricted in the middle. Hind legs longer than others. [In the male abdomen ('belly') tapers posteriorly.]

Behaviour of adults

(a) *Attraction of adults to coloured lights.*—According to Lloyd adults of *A. vaporariorum* are attracted to yellow-coloured light. A similar study was carried out with *B. gossypiperda* and it was found that they showed the greatest attraction for yellow-green coloured light and next highest for yellow, the least number of them was attracted to bright red, orange red, dark green and purple coloured lights.

(b) *Range of flight.*—*B. gossypiperda* feeds on a large number of plants. It is, therefore, difficult to study its range of flight unless the locality in which such observations are made is free from this pest. Observations, however, were made to determine the height which this pest attains during flight. The insect has been found at a height of 40 feet above ground level but as it is carried away long distances by wind, it is not improbable that it may be found at heights greater than that recorded by us.

Life-history

Bemisia gossypiperda produces about 12 broods in a year but the generations overlap, and, therefore, all stages of the pest are met with throughout the year. It breeds practically all the year round, often parthenogenetically, the unfertilized eggs producing only males. Eggs are laid singly on the leaves and each is inserted in the tissue by a short stalk. In confinement they may also be laid on the stumps of defoliated seedlings. In this case the nymphs die off shortly after hatching. Laboratory observations show that the top and middle leaves are preferred for oviposition, for 51.5 per cent and 46.7 per cent respectively of the eggs were laid on them, while 1.8 per cent only were laid on the lower leaves. Much the same kind of behaviour is also noticed in the field. It has also been observed that in nature eggs are laid invariably on the lower side of a leaf.

A single female may lay, on an average, 28 to 43 eggs during its oviposition period, which may last from 2 to 18 days: she lays six to eight eggs in 24 hours. Temperatures between 33 and 37°C. are the most suitable for oviposition, no eggs were laid at 19°C.

The incubation period varies from 3 to 33 days depending upon the temperature. During the season of the growth of cotton plants, i.e. April to September, eggs hatch in three to five days, during October to November in 5-17 days and during February to March in 7 to 16 days. In December and January the incubation period may be as long as 33 days.

The insect has three nymphal and a pupal instar. The duration of the three nymphal instars varies from 8 to 14 days from April to the end of September but from October onwards this period is considerably prolonged and ranges from 17 to 73 days. Unlike the Citrus *Aleurodidæ* the pupal stage of *Bemisia gossypiperda* is very short and occupies only two to eight days. The adults, which emerge, as a rule, during the day time, do not live very long in summer and in captivity their life lasted two to five days. During November, however, some adults lived up to 24 days. A complete life-cycle from egg to adult may occupy from 14 to 107 days. During April-September it occupied only 14 to 21 days. The shortest life-cycles were observed during August. From October onward the life-cycle is much prolonged and in one case during these investigations it extended up to 97 days between November-February.

Food plants and seasonal history

Bemisia gossypiperda is polyphagous and a list of its host plants in the Punjab includes no less than 44 species, both cultivated and wild, belonging to about 13 families. Irrespective of any preference for any of its food plants, the density of white-fly population on a particular host is influenced to a large extent by its proximity to the cotton fields infested by it, protection from wind, adequate humidity and, what is probably most important of all, radical changes in the composition of cell-sap of the leaves of the plant at different periods of growth indicating the physiological state of the host. At times, however, the intensity of infestation on certain of the host plants increases sporadically.

In general the white-fly undergoes three phases of migration during the course of a year :

1. During November when the cotton crop is maturing and the leaves are drying up and shedding, the white-fly population falls on this plant and infestation starts on such alternative host plants as rape (*Brassica napus*), cauliflower (*B. oleracea*), turnip (*B. rapa*) and potato (*Solanum tuberosum*), among the cultivated plants and *Sonchus* spp., *Euphorbia* sp. and *Convolvulus arvensis* among the commoner of the weeds. From December onwards the number of adults falls considerably, but the immature stages remain on these alternative hosts throughout the winter. The adults commence emerging from about the end of January and multiply once again on the winter host plants already named above.

2. By the end of March the white-fly migrates to its spring hosts, namely, *Cucumis melo*, *Citrullus vulgaris*, *Cucumis sativus*, *Lagenaria vulgaris*, etc. where rapid multiplication takes place. From April onwards ratoon cottons, *Nicotiana tabacum*, *Hibiscus esculentus* and *Althea rosea* are also severely infested. During April and May ratoon cottons and melons form the most important breeding centres.

3. The white-fly makes its appearance on the new cottons early in May soon after the crop has germinated, but the attack at this stage is extremely low as compared to the other host plants. From June, however, partly because of migration from the alternative host plants, but mainly on account of the rapid multiplication of the pest, the intensity of attack increases enormously on the new cotton crop. The data collected have established the fact that the period of the severest attack on cottons extends from June to August

after which the infestation, as a rule, declines abruptly. To determine the status of various host plants as the true food-plants of *B. gossypiperda*, a series of cross-inoculations was carried out. It was found that this insect is not so unorthodox as to feed on all and sundry plants. For example, repeated attempts were made unsuccessfully to breed it on *chari* (*Andropogon Sorghum*). Further, it has been observed that the pest prefers certain plants for oviposition and this preference depends upon the time of the year and its own period of migration.

Comparative infestation of different varieties of cotton

It has been stated commonly that the incidence of white-fly attack is higher on the broad-leaved varieties (Punjab-American) than on the narrow-leaved varieties (*desi*). A census of white-fly nymphs and eggs taken on four varieties, 289-F and 4-F, representing the Punjab-Americans, and *mollisoni* and *sanguineum*, representing the *desi* varieties, was taken from 1931 to 1933. The results indicate that the insect shows no selective preference in infesting varieties of cotton all of which are liable to be attacked almost equally severely. The *Desi* varieties, in general, are comparatively more infested during the growing period, i.e. till about the end of August, after which the attack increases on the American varieties. The attack may increase once again on *desi* varieties towards the end of the season when they may begin to sprout.

During 1934 and 1935 some new selections of the American types were compared with *mollisoni*, 4-F and 289-F. During the growing period the white-fly infestation was significantly higher on the *desi* variety, whereas during the fruiting period it increased on the American types. While determining the cause of this change-over of infestation, it was noticed that the incidence of attack corresponded with the trend of the pH curve, indicating partiality towards higher values. The infestation, however, was not affected immediately, but after some time, because the nymphs, being fixed on the leaves, must take some time before the effect of the change in pH can be appreciated by them. This finding is of considerable importance in absolving this pest of the blame of being the main cause of cotton failures because it is only the Americans that fail.

Incidence of white-fly attack in relation to amount of water applied

The type which received the largest number of irrigations and, consequently, the maximum amount of water, had on an average, the lowest white-fly attack. On the other hand, the types which received restricted irrigations with a corresponding minimum supply of water, were comparatively severely infested.

Influence of date of sowing and of manures on the incidence of white-fly attack

The incidence of attack on the early-sown crop was found to be comparatively higher up to September after which it was practically uniform on all the sowings. There is not much difference between the cotton sown between 1st May and 1st June, but the cotton sown later escapes the attack of the pest. The intensity of attack was slightly lower on manured plants but plants treated with ammonium sulphate or super phosphate early in the season (June

or July) at the rate of 1.5 maunds per acre showed comparatively less attack. It was also observed that the relative infestation was significantly lower in the plots treated early with nitrogenous manures both in the rich and poor soils. On the other hand, early manuring in poor soils and late manuring in rich soils yielded better out-turns.

Climatic conditions and condition of the host plant and white-fly attack

A correlation between the white-fly population and the meteorological condition of a locality points to the conclusion that the attack is highest in areas (canal colony tracts) of high temperature and scanty rainfall. On the other hand, the attack is lowest in the south-east and the north-west Punjab where rainfall is high or the climate is rather temperate.

Some experiments were also performed to study the condition of the host plant, as influenced by changes in the soil, in relation to its infestation by the white-fly. Although very definite conclusions are not possible, the evidence available seems to show that white-fly attack was relatively low on plants grown in soils of slightly lower pH value (6.64 brought about by addition of ferrous sulphate) as compared to those grown on more alkaline soils (8.55; treatment with sodium carbonate). The vegetative growth of the plants may indirectly affect infestation. With ferrous sulphate treatment, however, the attack was comparatively lower although the plants were below normal in their vegetative growth. Further, the highest number of bolls and relatively low shedding were noticed on the plants treated with ammonium sulphate or sodium nitrate. The least alkaline soils gave very poor yields in spite of low attack of the white-fly, but the highly alkaline soil also gave a poor yield with a correspondingly higher infestation.

Although there seems some possibility of preventing an attack of sucking insects through soil treatment, the full possibilities of controlling this pest by this method remain unexplored.

Nature and extent of damage

Unlike most sucking insects, *Aleurodidæ* do not produce any visible injury, such as spotting, crinkling or any other deformation on their host plants. *Bemisia gossypiperda* has been regarded as the vector of leaf-crinkle of cotton in Sudan and a probable transmitter of leaf-curl in *Zinnia* at Dehra Dun and of tobacco at Pusa. In the Punjab up to the present time the white-fly infestation of cotton is not associated with leaf-crinkle of any type whether caused by a virus or any other toxic agency. Indeed, leaf-curl or leaf-crinkle of cotton as a virus disease has not so far been discovered in these parts. Nor is there any evidence that the white-fly of cotton is in any way associated with the recently discovered 'smalling' disease of cotton in the Punjab.

The obvious results of white-fly attack are: (1) drain of the plant juices, which results in lowering the vitality of the plants, (2) development of a black fungus on the honey-dew which is exuded by the nymphs and pupæ and drops down to the lower leaves, thereby interfering with the photosynthetic activities of the plant.

In the absence of any mechanical injury to the plant tissues the effects of white-fly infestation were studied in relation to the physiological changes connected with the growth and reproductive activities of the host plant.

The work carried out at Lyallpur throughout the period of this scheme has shown that the infestation of a plant by the white-fly is detrimental in all its stages, viz. the growth period, the time of flower and boll formation and of lint and seed development. Further, the effects of the attack are more serious in the later part of the growing period—the flowering stage, when important changes and adjustments in the vital nutrients are going on in the tissues of the plants and making it most susceptible to injury.

During the period of severe infestation the vegetative growth is checked and in most serious cases may almost be stopped. Boll formation becomes indirectly proportional to the intensity of attack; whereas the shedding and bad opening remain in direct proportion. The bolls produced by the uninfested plants were well-developed and yielded a maximum weight of *kapas*. The severity of infestation, particularly when it appears late in the growing season, lowers the yield of lint and in all respects affects the plants adversely.

An analysis of the above conditions has shown that infested plants are deficient in their moisture percentage with a corresponding increase in dry matter. Attacked plants also carry a higher carbon-nitrogen ratio, a condition which has been shown to retard both vegetative and reproductive growth. Experiments performed in 1932, 1934 and 1935 definitely indicated that a relatively higher infestation by the white-fly not only decreases the dry weight of the plant, but also leads to slightly greater shedding of leaves, floral buds, flowers, etc. It was also determined that the maximum loss in the dry weight of the vegetative portion takes place during August-September. From October, however, the loss is manifested in the weight of the reproductive organs.

Mineral ash.—About 36 per cent more ash was produced by the healthy plants of which 11.3 per cent was estimated to have been transported to the bolls.

Fat.—The percentage of fat was comparatively higher in the foliage of healthy plants, while that of carbohydrates was relatively higher in the foliage of infested plants throughout the season.

Analysis of healthy and infested plants in 1932 showed that (1) nitrogen is higher in the foliage of the uninfested cotton plants till the middle of August after which it may rise in the foliage of the infested plants: it is considerably higher in the bolls of the uninfested plants, (2) healthy plants, on the whole, produce more total nitrogen than the infested ones in which the transport of nitrogen, ash and fat from the vegetative to the reproductive organs is markedly reduced. It is surmised, therefore, that the reduction of bolls which occurs on the infested plants is the result of some dislocation in the carbohydrate and protein balance.

Control

Predators and parasites.—The third instar nymphs and pupæ of *Bemisia gossypiperda* have been found parasitized by Chalcid parasites which deposit

their eggs within the body of their hosts, the parasites completing their life-cycle in six to seven days in August. Larvæ of a lacewing fly (*Chrysopa* sp.) and of a Coccinellid beetle (*Brumus*) have been observed feeding on the adults of the cotton white-fly and observations also showed that the number of adults killed by an individual grub of *Brumus* or *Chrysopa* sp. far exceeds that which is actually required for its feeding. These enemies, however, do not afford any satisfactory control.

Cultural methods.—The cultural practices, such as altering the dates of sowing and the amount of water applied, do not hold out much promise of materially reducing infestation by this insect. Clean cultivation and safe disposal of alternative hosts in the cotton off-season will, however, to a certain extent reduce the extent of white-fly attack. Proper manuring at the right time may help the plants to recover from the damage caused by the white-fly.

Spraying.—Small-scale spraying experiments were conducted against this pest during the years 1929-1932. Results of single and double sprayings were compared. Double sprayings once in July and again in August proved very effective. A single spraying in September or double spraying with the 2nd treatment falling during that month resulted in a relatively much lower increase than even in case of a single spraying in July or August. Late spraying in September causes additional flower and boll shedding and ultimately affects the yield adversely. Since these spraying operations gave encouraging results, it was felt necessary to estimate the efficacy and economics of this method of control on a field scale. This was made possible by a grant of the Indian Central Cotton Committee for spraying trials during 1933.

Extensive spraying operations were conducted from the 10th July to 31st August on the British Cotton Growing Association Farm, Khanewal, and at the Military Farms, Okara. These spraying trials were repeated during 1934 to confirm the previous results and to improve the technique of spraying. Both *desi* and American cottons were treated in 1934, when the operations were extended to Sargodha as well. Most of the area under these field trials was sprayed with rosin compound but a number of other insecticides, such as rosin soap, fish-oil soap, tobacco decoction, kerosene oil emulsion and lime sulphur, were tested on smaller scales. Rosin compound proved to be the most satisfactory insecticide.

In all 1873 acres of cotton crop were sprayed during 1933 and 2640 acres in 1934. Machines of the type of cart sprayer proved useful as their working was very easy and economical, but the Hardie sprayer with two horse power motor pump yielded very satisfactory results, both with regard to the mortality of the pest and the cost of spraying. These cart sprayers can only be worked when the crop is sown in lines and has not grown very tall. To test the possibilities of employing a sprayer which kept outside the field—an orchard power sprayer—with pipes laid in the field was used. It gave a kill of 95.7 per cent of adults and 93.9 per cent in nymphs and pupæ—the maximum obtained, but it was cumbersome and uneconomical.

The maximum quantity of insecticide consumed in 1933 was 58.2 and 43.3 gallons per acre with Hardie and Sapom respectively: the orchard power sprayer consumed 68.4 gallons per acre at Okara.

The average time required for spraying one acre of cotton was 12·4 to 26 minutes, depending upon the machine.

The cost of spraying with rosin compound varied on the whole, from Re. 1-2-3 per acre with the Sapom sprayer to Re. 1-7-9 with the Hardie sprayer and Rs. 2-9-9 with the orchard power sprayer during 1934. These costs were lower than those of 1933 by 15·5 per cent, 8·1 per cent and 21·1 per cent respectively. The cost was comparatively higher with other insecticides under trial. It is possible, however, to reduce the cost still further by paying more attention to details connected with these operations.

Spraying *desi* cotton during the month of July and American cotton during August increased the yield. The average increase in yield per acre by spraying was 0·5 to 2·0 mds. for American when sprayed in July and August and about the same for *desi* when sprayed in July. Spraying of *desi* cottons in August, however, yielded negative results. It was found that spraying must be done before the flowers appear.

A NEW PEST (*ACANTHIOPHILUS HELIANTHI* ROSSI, TRYPETIDAE) OF SAFFLOWER IN INDIA

BY

HEM SINGH PRUTHI, M.Sc., Ph.D. (CANTAB), F. N. I.

Imperial Entomologist

AND

H. L. BHATIA, M.Sc.

Imperial Agricultural Research Institute, New Delhi

(Received for publication on 24 August 1939)

(With Plate III)

INTRODUCTORY

ABOUT the middle of March, 1939, in the experimental plots of safflower (*Carthamus tinctorius*) of the Imperial Economic Botanist at New Delhi, some of the flower buds presented a diseased appearance, the chief symptom of which was an odorous juice oozing out from the apical region of the buds. Such buds were found to contain dirty-white maggots of a fruit fly which has been identified as *Acanthiophilus helianthi* Rossi. This is the first record of this genus and species from India.

The pest was very active during March, April and May and caused serious damage in both early and late sown varieties of safflower, the infestation being as high as 90 per cent. The young florets were damaged by the maggots with the result that the buds opened partially or did not open at all.

As the cultivation of safflower is being considerably extended in India for the dye obtained from its flowers and for the oil obtained from its seeds, there is danger of the spread of the pest which can be carried in the pupal stage mixed amongst the seeds from one place to another.

Detailed observations on the biology of the pest have been taken during the last spring and summer. As the pest is new and is of considerable potential importance, the results of investigation so far obtained are being published, so that workers in other regions of India may be able to recognize the pest if it occurs there.

DISTRIBUTION AND HOST PLANTS

Chiefly through the courtesy of Mr. Munro, Entomologist, Department of Agriculture, South Africa, we have been able to obtain the following information about the distribution of *Acanthiophilus helianthi* Rossi in other countries :—

The species was originally described by Rossi in 1790 and has been referred to in literature as *Trypeta eluta* by several workers [Meigen, 1826 ; Efflatoun

1924, etc.]. The species is recorded from the Canary Islands, the Mediterranean region, Central Europe, North Africa, Egypt, the Sudan, Erytrea, Asia Minor, Persia and Central Asia. The recorded host plants are: *Centaurea* spp., *Cnicus lanceolatus*, *Silybum marianum*, *Onopordon illyricum*, *Amherboa lippi*, *Leuzea conifera* and *Carthamus tinctorius*. According to Hendel [1927], the maggots as a rule live in the flower heads, producing galls, but they are also found at times in the stems, as in the case of *Cnicus lanceolatus*. Most of the host plants listed above seem to be more or less weeds, with the exception of *Carthamus tinctorius* (safflower), with regard to which Hendel [1927], reports a record by Handlirsch of rearing the maggots from the flower head but does not state the locality.

As stated already, there is no previous record of the occurrence of *A. helianthi* from India, but probably the fly recorded attacking seeds in plants of safflower [Rati Ram, 1927] in the Central Provinces was this species.

NATURE OF DAMAGE

The flies were observed on wing in the field for the first time about the middle of March and infestation of the safflower buds by the maggots was evident a week afterwards. The maggots feed upon the essential organs of the florets and even bore into the thalamus. The infested bud begins to rot and the fluid thus produced oozes out from its apical portion and gives it a damp appearance (Plate III, fig. 2). If such a bud is squeezed between the fingers, the fermented liquid along with one or two maggots come out of the tip of the bud. Furthermore, in advanced stage of attack, the florets become black, presenting an emaciated and withered appearance (Plate III, fig. 3.)

INCIDENCE OF THE PEST IN VARIOUS VARIETIES

Out of the 34, varieties grown for experimental purposes by the Imperial Economic Botanist, the felted varieties (No. 11—15) and the varieties No. 20-22, 30 and 34 were attacked more than the rest, early in the season, viz., from the third week of March up to the end of the first week of April. All the varieties named above are much less spiny and are late in flowering. The second generation of the pest which lasted during the second and third weeks of April was rather small in numbers, probably on account of the activity of the parasites and the predator described hereafter. In the fourth week of April the third generation of the fly was in evidence and the incidence increased rapidly in all the varieties, the degree of infestation being again higher in the felted and the less spiny varieties as compared to the spiny ones. A statement of the attack of the pest in different varieties during the season would be found in the appendix.

It may be added that incidence in the wild safflower which is very spiny was lower than in any of the spiny cultivated varieties. However, after the first week of May when all the cultivated varieties had been harvested, the pest was found in abundance in the wild safflower, which was in flower and was found growing along the field drains and elsewhere in uncultivated areas.

LIFE-HISTORY AND DESCRIPTION OF VARIOUS STAGES

The female fly deposits her eggs in the field from sunrise till sunset. She is provided with a long proboscis, which she uses to probe a number of places with its tip, and rather than depositing the eggs in each probing being, she moves on to the next place. The behaviour of the female in this respect is very different in several respects from that of the house fly (*M. domestica*). During this process which is called oviposition, she frequently bends the abdomen, its posterior end reaching to the surface of the soil. It is entirely unobscured by the wings. **PLATE III**

- FIG. 1. Healthy bud (Natural size) with its hind pair of legs before it is put in the soil.
- FIG. 2. Damaged bud (Natural size) after it has been put in the soil.
- FIG. 3. Vertical section of damaged bud (Natural size) showing the position of the egg mass.
- FIG. 4. An egg mass ($\times 4$).
- FIG. 5. The egg ($\times 16$).
- FIG. 6. The hatching out maggot ($\times 16$).
- FIG. 7. The newly hatched maggot ($\times 16$).
- FIG. 8. The 'Full-grown maggot' ($\times 10$).
- FIG. 9. The anterior spiracle of one side ($\times 192$).
- FIG. 10. The antenna and maxillary palp of one side (highly magnified).
- FIG. 11. The cephalo-pharyngeal skeleton ($\times 80$).
- FIG. 12. The posterior spiracles ($\times 100$).
- FIG. 13. The pupa ($\times 10$).
- FIG. 14. The female fly ($\times 10$).
- FIG. 15. The head of the imago ($\times 10$).

Each spiracle has two slits.

The young maggot is 5.0 mm. \times 1.5 mm. It is a small, white, worm-like creature, with a head and a tail, tapering towards the head. The posterior end of the maggot is slightly enlarged. The head is provided with the oral opening situated on the dorsal side. A pair of antennae, each antenna being composed of three segments, is minute, cylindrical and has a knob at the end. The maxillary palps, each palp is situated just behind the antennae and has three to four, parallel, sensory bristles (Plate III, fig. 10, m. p.); (iii) the cephalo-pharyngeal skeleton, which is highly sclerotized and extends up to the first thoracic segment (Plate III, fig. 11). It is composed of the following:—

(i) A pair of mandibles or mandibular sclerites or mandibular hooks, which are furnished each with two strong teeth, the apical tooth is curved and the pre-apical tooth, which is smaller and less curved, is directed backwards and somewhat downwards.



- (2) A pair of hypostomal or intermediate sclerites (*h*). They are elongated, the broad posterior margin being one and a half times or twice the narrow anterior margin. A pair of rod-shaped sub-hypostomal sclerites (*s. h.*) connects these sclerites with the oral hooks.
- (3) Cephalo-pharyngeal sclerite (*Cph.*) which is made up of two shafts united anteriorly, the free portions being forked. Posteriorly, each branch of the fork is further divided into two appendages.

The body of the full-grown maggot has besides the head, 11 visible segments, three thoracic and eight abdominal. At this stage the maggot is amphipneustic, the anterior and posterior spiracles being borne by the first thoracic and the last body segment respectively (Plate III, figs. 8, 9, 12). The long tracheal tubes are visible within the integument. The anterior spiracles are cup-shaped, the margin of each spiracle being fringed with six oval lobes. The posterior spiracles are almost reniform, each possessing three elongated oval slits which are notched at the peripheral end. The inner walls of the slits are chitinized and fimbriated. In each of the inter-spiracular areas there are one to four very minute hyaline lanceolate processes. The pseudopods, which are clearly seen in the abdominal region of the maggots of several species of fruit flies especially of the subfamily Dacinae, are not distinct in this species. Girdles of four to eight rows of minute conical spines with their apices directed backwards are found in the inter-segmental region of the body. There are fewer rows of spines in the thoracic than in the abdominal segments. The maggot is not capable of jumping, a habit which is so characteristic of the maggots of other Trypetid flies, particularly those infesting fruits.

Pupation generally takes place in the flower bud, the puparia being rarely met with in the soil. The puparium (Plate III, fig. 13) measures 4.25 mm. \times 1.75 mm. It is barrel-shaped, black with a metallic tinge. There is a small depression in the thoracic region. The larval girdles of spines and the spiracles are retained and occupy the same positions as in the maggot.

The flies emerge and leave the infested flower bud through small holes on its surface, previously made by the full-grown maggot, each exit hole being about 2 mm. in diameter. The newly emerged flies are rather sluggish, their eyes emerald-green (Plate III, fig. 15) and the body ash-grey. The greyish markings on the apices of wings are not well developed. The flies soon become active and resume their normal appearance within a few hours in the field. They are ash-coloured, bristly, with reddish-brown frons and light brown legs. The male is smaller in size than the female (Plate III, fig. 14) which is about 6.5 mm. in length. The flies are lovers of sunshine flying about flower heads of safflower from sunrise to sunset.

The duration of various stages (average of five readings) in April in the laboratory having average maximum and minimum temperatures of 85.2°F. and 78.5°F, respectively, was as follows: egg, 25 hours; maggot and pupa, each seven days. The adults could be kept alive when fed on peptone, yeast and sugar under laboratory conditions for about ten days but one male specimen lived as long as five weeks. During the period of six weeks from about the middle of March up to the first week of May in the safflower season the fly completes three generations,

PARASITES AND PREDATORS

The following parasites and predators of the pest were observed :—

1. *Tropideucoila* sp. (probably a new species of the family Cynipidæ). The adults of the parasite were found in small numbers in the field between the end of March and the second week of April. Also a few specimens emerged from the rearing cages in the laboratory. The parasite did not seem to appreciably reduce the population of the pest.

2. *Ormyrus* sp. (Fam. Torymidæ). The chalcidoid wasp was very common in the field and emerged in large numbers in the rearing cages in the laboratory during the second and third weeks of April when the pest was in the second generation. On account of parasitization by this species a significant reduction in the population of the pest was observed in the field and the attack decreased markedly as would be found in the statement of incidence of the pest in the appendix. In association with this parasite, the species *Stenomalus muscarum* (Linn.) (Pteromalidæ) and *Eurytoma* sp. were also found, but the precise role played by them has not been determined. About the same time, the neuropterous predator, *Chrysopa virgestes* was also quite common in the field and its nymphs were found devouring the pest maggots. In the third generation of the pest, extending from the last week of April up to the second week of May, the chalcidoid parasites and the predator were found occurring in very small numbers and the higher incidence of the pest at this time was probably due to this.

SUMMARY

1. *Acanthiophilus helianthi* (Trypetidæ) has been noticed for the first time as a serious pest attacking safflower in India. This is also the first record of this genus and species from this country.

2. The pest was active from March to May and caused serious damage to both early and late sown varieties of safflower, in some of which the infestation of floral buds, was as high as 90 per cent.

3. The fly punctures the flower bud and lays eggs on the inner side of its involucre. The maggots feed upon the florets and even bore into the thalamus. The florets are thus partially or completely destroyed, with the result that the bud does not open. Pupation takes place generally in the bud. The flies emerge through small holes on the surface of the bud. The duration of various stages in April in the laboratory with average maximum and minimum temperatures of 85·2°F. and 78·5°F. was : egg, 25 hours, maggot and pupa, each seven days and adult, 10 days. The fly has three generations during the season (March-May).

4. Two species of hymenopterous parasites on the immature stages of the pest are recorded.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. H. K. Munro, Entomologist, Department of Agriculture, Union of S. Africa, Pretoria, for the identification of the species and for information about its distribution and host plants outside

India. We are also thankful to the Imperial Economic Botanist for the facilities provided for examining his experimental crop of safflower for the study of the pest. To Mr. Deshpande, M.Sc. (Ag.), Special Research Assistant of the Botanical Section, we are obliged for supplying some particulars of the varieties.

REFERENCES

- Bhatia, H. L. and Singh, M. (1939). *Ind. J. Ent.* **1**, 110-11
 Efflatoun, H. C. (1924). *Mem. Soc. Roy. Ent. Egypt* **2**, 117
 Meigen, J. W. (1826). *Systematische Beschreibung europaischen Zweiflgüligen Insekten*, **5**, 344
 Ram, R. (1917). *Proc. Ent. Mtg. Pusa* **2**, 97
 Rondani, C. (1871). *Dipterologiae Italicae Prodromi* **7**, 12
 Rossi, P. (179). *Fauna Etrusca* **2**, Liburni
 Seguy, E. (1930). *Mem. Soc. Sci. Nat. Marocco* **24**, 177

APPENDIX

Statement of the incidence of the fruit fly on various varieties of safflower at Delhi from 28th March to 15th May 1939

Serial No. and features of the type	Number of observations	Total No. of flower buds examined	Average percent-age of attack	Range of incidence and remarks
1. (S. L.)	17	449	14.7	5—40 per cent; attack increased after the third week of April.
2. (S. L.)	17	500	12.4	5—45 per cent; attack increased after the third week of April.
3. (S. E.)	17	415	16.6	5—45 per cent; attack increased after the third week of April.
4. (S. E.)	17	478	14.6	5—35 per cent; attack increased after the third week of April.
5. (S. L.)	15	431	16.9	5—45 per cent; attack increased after the third week of April.
6. (S. L.)	15	471	14.8	5—45 per cent; attack increased after the third week of April.

S = Big spines

L = Late variety

E = Early variety

APPENDIX—*contd.*

Serial No. and features of the type	Number of observations.	Total No. of flower buds examined	Average percentage of attack	Range of incidence and remarks
7. (S. L.)	15	437	16.4	5—50 per cent; attack increased after the third week of April.
8. (s. L.)	16	464	15.5	10—45 per cent; attack high up to the first week and after the third week of April.
9. (s. L.)	16	555	10.5	5—50 per cent; attack increased after the third week of April.
10. (s. L.)	16	471	10.3	5—35 per cent; attack increased after the third week of April.
11. (s. L. F.)	16	376	50.0	10—70 per cent; attack high up to the first week and after the third week of April.
12. (s. L. F.)	16	445	31.2	10—70 per cent; attack high up to the first week and after the third week of April.
13. (s. L. F.)	16	569	19.5	10—65 per cent; attack high up to the first week and after the third week of April.
14. (s. L. F.)	16	424	16.4	5—45 per cent; attack high up to the end of March and after the third week of April.
15. (s. L. F.)	16	490	15.7	5—55 per cent; attack high up to the first week and after the third week of April.
16. (s. L. F.)	16	417	21.1	5—60 per cent; attack increased after the third week of April.
17. (s. L. F.)	16	497	13.8	5—65 per cent; attack increased after the third week of April.
18. (s. L.)	15	319	17.8	5—45 per cent; attack increased after the third week of April.

S = Big spines

s = Small spines

L = Late variety

F = Felted variety

APPENDIX—*contd.*

Serial No. and features of the type	Number of observations	Total No. of flower buds examined	Average percentage of attack	Range of incidence and remarks
19. (s. L.)	15	332	14.7	5—40 per cent; attack increased after the third week of April.
20. (s. L.)	15	360	31.3	5—65 per cent; attack high up to the first and after the second week of April.
21. (s. L.)	15	349	35.5	25—55 per cent; attack high throughout but more so after the third week of April.
22. (s. L.)	15	341	30.7	10—60 per cent; attack high up to the first week and after the third week of April.
23. (s. L.)	15	357	18.2	5—45 per cent; attack increased after the third week of April.
24. (s. L.)	15	332	11.4	5—30 per cent; attack increased after the third week of April.
25. (s. L.)	15	366	17.4	5—40 per cent; attack increased after the third week of April.
26. (S. E.)	15	312	20.8	5—50 per cent; attack increased after the third week of April.

S—Big spines

s—Small spines

L—Late variety

E—Early variety

APPENDIX—*contd.*

Seiral No. and features of the type	Number of observa- tions	Total No. of flower bunds examined	Average percent- age of attack	Range of incidence and remarks
27. (S. E.)	15	338	20.1	5—50 per cent; attack increased after the third week of April.
28. (S. E.)	15	378	13.7	5—60 per cent; attack increased after the third week of April.
29. (s. E.)	15	318	30.1	5—60 per cent; attack increased after the third week of April.
30. (s. L.)	15	347	61.9	30—95 per cent; attack high throughout but more in the beginning and towards the end of the season.
31. (s. E.)	14	350	26.8	10—55 per cent; attack increased after the third week of April.
32. (s. E.)	14	329	31.0	10—60 per cent; attack increased after the third week of April.
33. (s. E.)	14	335	31.6	5—75 per cent; attack increased after the third week of April.
34. (s. E.)	14	359	54.5	40—85 per cent; attack high, more in the beginning and towards the end of the season.

S—Big spines

s—Small spines

L—Late variety

E—Early variety

MICRO-BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS

I. CHANGES IN THE CONSTITUENTS OF RICE STRAW (KANAK-TARA) PRODUCED BY MICRO-ORGANISMS PRESENT IN SOIL SUSPENSION UNDER AEROBIC, ANAEROBIC AND WATERLOGGED CONDITIONS

BY

S. S. GUHA SIRCAR,

S. C. DE

AND

H. D. BHOWMICK

Department of Chemistry, University of Dacca, Dacca

(Received for publication on 14 December 1933)

(With one text figure)

THE disintegration of various organic residues under the influence of micro-organisms under aerobic, anaerobic and waterlogged conditions have been investigated by various workers. The decomposition of organic matter in well-aerated soils takes place quite rapidly, and Boussingnault [1853] was the first to show that the process was mainly an oxidation, oxygen being absorbed and an approximately equal volume of carbon dioxide being evolved. On the other hand, it is well known that in badly-aerated waterlogged soils, the destruction of organic matter is slow and incomplete and leads mainly to a reduction to simple organic compounds like methane, hydrogen, organic acids, alcohols, etc. The conditions that control the course of such decompositions have been worked out by the careful and painstaking investigations of Omeiiansky [1895, 1897], Hebert [1892], Deherain [1884, 1888, 1902], Hoppe-Seyler [1899], and more recently by Waksman and others [1925-31], Page [1932] and others. The anaerobic type of decomposition has been studied by Deherain [1884, 1888, 1902] and Omeiiansky [1895, 1897], who showed that the micro-organisms require nitrogen salts for speedy destruction of organic matter. Waksman and his co-workers [1925-31] and Anderson [1926] confirmed this finding and established a direct relation between the amount of cellulose or hemicellulose decomposed and the nitrogen salt converted into organic form (microbial protein), a 30 : 1 ratio being found to be suitable in a number of cases where the effect of cellulose added to soil was studied. A constant synthesis of proteins and other complex nitrogenous materials by the microbes in soil was noticed even earlier by Deherain [1902] and Lathorp [1912, 1916, 1917].

In the presence of sufficient nitrogen, the decomposition of cellulose and hemicelluloses in the pure state, or in plant residues, by soil organisms takes place rapidly. Waksman and his co-workers [1925-31; 1936] showed clearly that if the nitrogen content of the medium is high, ammonia is set free and if it is low, either the decomposition is slowed down or is only hastened

by adding inorganic nitrogen salts up to a concentration of 1.7 to 1.8 per cent of the material. Acharya [1935] showed that under anaerobic conditions much less nitrogen was required than under aerobic conditions.

It has also been shown by Waksman and others [Waksman and co-workers, 1927-31; Deherain, 1902; Hebert and Heim, 1911; Hebert 1892; Hoppe-Seylor, 1899; Rege, 1927; Bach, 1926; Egorov, 1911; Konig, 1926; Rose and Lisse, 1917; Bray and Andrews, 1924] that of the various components of plant organic matter, water-soluble materials are utilized with the greatest speed followed by the hemicelluloses and pentosans, then by cellulose and ultimately by lignin. Some hemicelluloses and pentosans may, however, be re-synthesized by the organisms as well as considerable amounts of proteins. The dark residue or the so-called humus resulting from the decomposition consists, therefore, mainly of modified lignin complexes of plant origin, microbial proteins and hemicelluloses, partly of plant origin and partly synthetic, together with a small amount of fatty and waxy substances, chitinous material, etc.

It has now been established that the course and extent of decomposition of plant residues are influenced by the nature and composition of the material, the degree of aeration, moisture supply, temperature, pH of the medium, nature of the micro-organisms attacking the residues, etc. The general stability of lignins explains the lower velocity of decomposition of fibres, like wood and jute, in which the cellulose is associated with lignin, cutin or pectin [Langwell and Lymn, 1923, 1932; Fowler and Joshie, 1920; Waksman and co-workers, 1927-31]. Isolated lignin has been found to be even more stable and has been regarded as bacteriostatics [Bosuff and Bushwell, 1929, 1930, 1934; Phillips and co-workers, 1930; Waksman and others, 1925-31; 1936.]

Waksman and Tenney [1927-1930] have also shown that a young plant with higher content of water-soluble fraction and nitrogen and lower amounts of lignin and hemicelluloses, etc. decompose much more quickly than mature residues in which the conditions are reversed. The nitrogen content is shown to be a powerful deciding factor. Decomposition of plant materials poor in nitrogen leads to a relative and absolute increase of crude protein content, whereas those rich in nitrogen lose the excess of the element in the form of ammonia.

It has been shown by various workers [Waksman and Tenney, 1929, 1930; Waksman, Tenney and Stevens, 1928 and Acharya, 1935] that the rate of decomposition of plant residues as a whole as well as of its various constituents is greatest under aerobic, intermediate under waterlogged (partially aerobic), and least under anaerobic conditions. Again, whereas nitrogen supply is of paramount importance under aerobic conditions, it is of less importance under anaerobic conditions as the requirement of nitrogen by anaerobic organisms is very low. This is well illustrated in Acharya's experiments [1935]. During the decomposition of rice straw under aerobic, anaerobic, and waterlogged conditions, the protein content of the insoluble residue in the first case rises considerably, whereas in the latter two cases loss of this constituent was found to occur. Under mild aeration anaerobic condition of decomposition is stimulated, while strong aeration gives conditions resembling aerobic; waterlogged condition showing intermediate behaviour in all respects. The nitrogen relationship under the different treatments are of special interest. Similarly

the figures for 'nitrogen factor' [Rege, 1927] and 'nitrogen equivalent' [Richards and Norman, 1927] were shown to be the highest under aerobic (0.536 and 1.11, respectively), intermediate under waterlogged (0.395 and 0.961, respectively) and lowest under anaerobic conditions (0.069 and 0.169 respectively). The admission of even a limited amount of air in the anaerobic system at weekly intervals alters the nitrogen relationships, as shown by the synthesis of proteins from ammonia and by the rapid increase in the values for nitrogen factor and nitrogen equivalent.

Various investigations show that the temperature and reaction of the medium exert a profound influence on the rate and extent of decomposition of various organic materials by micro-organisms. Richards and Amoore [1920] at Rothamsted and Bushwell [1930] showed that the optimum conditions for the production of methane are: (1) a temperature between 35° and 40°C., (2) complete exclusion of air, (3) ample water-supply, (4) presence of some available nitrogen, and (5) absence of acidity. Similar results were also found by Acharya [1935] for anaerobic fermentation of rice straw. As the anaerobic decomposition is accompanied by the formation of various organic acids with the consequent lowering of pH of the medium, the fermentation process comes practically to a standstill as the pH falls to about 4.8, unless proper neutralizing agents are employed. Ammonium carbonate in sufficient amount was the most suitable neutralizing agent, while calcium carbonate, even in sufficient excess, was unable to retard the fall in the pH of the medium. When sodium nitrate was employed, there was no fall in pH but a gradual rise, accompanied by the maximum destruction of organic matter as a whole as well as of its various constituents. The reaction appeared to proceed in two stages: (1) denitrification and oxidation of organic matter, and (2) the development of alkalinity owing to the formation of alkali carbonates. This system resembled more the aerobic than the anaerobic system.

While the control of acidity is of great importance in the case of anaerobic fermentation, it is less so in the case of aerobic decomposition. In the latter case the fermentation is carried out by a larger variety of micro-organisms including bacteria, fungi and actinomycetes and thus the decomposition can proceed in neutral, slightly acid and slightly alkaline medium. Fungi, which are most important in the disposal of cellulosic materials under aerobic conditions are most active in slightly acid reaction. Too high an acidity or too high an alkalinity inhibit the action of both fungi and bacteria. Acharya [1935] found that there was no fall in pH of the medium, but a small rise, in the case of aerobic decomposition of rice straw, because the organic matter is converted completely to carbon dioxide and water without the formation of intermediate organic acids.

The aim of the present paper was to study the course of decomposition of two samples of rice straw at different stages of growth, under aerobic, anaerobic and waterlogged conditions with different nutrient solutions and under different pH of the media by following the changes in the amount of total organic matter as a whole as well as of its more important constituents with the progress of decomposition. At the same time attention was given to the changes in the pH of the media employed and to the influence of neutralizing

agents in successfully preventing the development of acidity. Stress was laid on the composition of the ultimate insoluble residue which would be expected to approach the composition of humus provided the decomposition went fast enough and far enough.

EXPERIMENTAL

Composition of plant materials used in the present investigation

The rice plants used in the present investigation were of two different ages, the rice straw No. 1 being younger, while the rice straw No. 2 was fully mature. Their proximate chemical compositions are given in Table I.

TABLE I

Proximate chemical composition of rice straw (Kanak Tara) used for decomposition studies

Chemical constituents	Rice straw No. 1 (young)		Rice straw No. 2 (mature)	
	100 gm. of original plant material	On per cent basis of dry material	100 gm. of original plant material	On per cent basis of dry material
Moisture ¹	12.99	..	12.45	..
Dry matter	87.01	..	87.55	..
Ash ²	13.12	15.08	10.15	11.60
Water-soluble fraction	16.46	18.92	12.46	14.23
Fats and waxes ³	1.32	1.52	0.74	0.85
Total pentosans ⁴	18.86	21.68	22.31	25.49
Crude cellulose ⁵	37.90	43.56	43.35	49.52
Ash in above	1.30	1.49	0.23	0.26
Pentosans in above	7.16	8.23	11.22	12.81
Cellulose	29.44	33.84	31.90	36.44
Crude lignin ⁶	20.68	23.78	22.83	26.08
Ash in lignin	6.38	7.33	5.10	5.83
Lignin	14.30	16.45	17.73	21.15
Total nitrogen ⁷	1.80	2.07	0.53	0.61
Crude protein ⁸	6.21	7.14	1.83	2.09

¹ Moisture—estimated at 105°C.

² Ash was found to contain Na, K, Ca, Mg, Fe, Al, Mn, SiO₂ and sulphate and phosphate, SiO₂ constituting the major part of the mineral constituents.

³ Fats and waxes—estimated by extraction with alcohol-benzene (1 : 1).

⁴ Pentosans—the standard Phloroglucide method as recommended by A. O. A. C. [1935] was employed.

⁵ Cellulose—the method of Norman and Jenkins [1935] was employed. The ash and pentosan content were subtracted to get the value for true cellulose.

⁶ Lignin—the method due to Ost and Wilkenig [1910] involving the use of 72 per cent H₂SO₄ gives results which are more consistent ; a modification of it put forward by Schwalbe [1925] was employed, the material being previously freed from fats and waxes and hemicelluloses (by extraction with hot 2 per cent HCl).

⁷ Total nitrogen—estimated by modified Kjeldahl method using Na₂SO₄, Na₂S₂O₃, salicylic acid and CuSO₄ in addition to conc. H₂SO₄.

⁸ Crude protein—the material was extracted by boiling water and analysed for nitrogen. The figure for nitrogen multiplied by 6.25 was expressed as crude protein.

The above data show that in the maturer plant, there are lower amounts of mineral matter (ash), fats and waxes, crude protein and water-soluble fraction, and higher amounts of cellulose, hemicelluloses and lignin. The water-soluble fraction on analysis was found to contain amino-acids and reducing sugars, the amount being greater in rice straw No. 1 than in rice straw No. 2. Similar results were obtained by Hunt [1889] and Waksman and Tenney [1927].

Decomposition experiments

The plant materials were cut into small pieces and then macerated in a pulverizing machine and 20 gm. of each variety were placed in a series of glass bottles. Mineral nutrients and soil inoculant* together with distilled water were added as described below and the contents well mixed so that the straw : water ratio was 1 : 10.

The aerobic decomposition was carried out in a series of glass bottles fitted with airtight corks through which two glass tubes (one reaching the bottom of the vessel and the other about two inches above the surface of the material) were inserted. Air passed through a wash-bottle containing distilled water was bubbled through the mass for about half an hour daily. It was observed that abundant fungal growth accompanied the process of decomposition under aerobic conditions. The mass of the residual matter became darker and darker with the progress of decomposition.

The anaerobic decomposition was carried in a number of 'reagent bottles' of about 300 c. c. capacity, fitted with rubber corks, exit tubes and pressure tubes about three inches long. The anaerobic condition was secured by a technique which would be clear from the diagram. The bottle (B) was connected as in the Fig. 1. The stop cock (a) was closed, (b) and (c) opened, and the enclosed air was removed by suction with a vacuum pump. The stop cock (b) was then closed and (a) opened and a measured volume of the solution (200 c.c.) was added to the enclosed straw and then (a) and (c) were closed. The end of the rubber tube was closed by a piece of glass tubing sealed at both ends. Acharya [1935] first filled the bottles with reaction mixture and then removed the air by suction. The method used in the present paper is easier and more convenient and involves no frothing up of the mixture during evacuation. The corks and rubber joints were coated with paraffin wax. The bottles were kept in a glass chamber at the laboratory temperature. The gases formed during fermentation were removed from time to time from the bottles by means of a Hempel gas burette, care being taken to avoid the entry of air. Qualitative analysis showed that the gases consisted mainly of carbon dioxide, methane, hydrogen and nitrogen depending on the nature of the mineral nutrients present in the medium.

The process of decomposition under waterlogged conditions was carried out in wide-mouthed bottles and the experiments were so arranged that the materials undergoing decomposition were completely covered with about two inches of water.

*Prepared by thoroughly agitating some well-manured garden soil with water, allowing the coarse particles to settle and centrifuging the supernatant liquid.

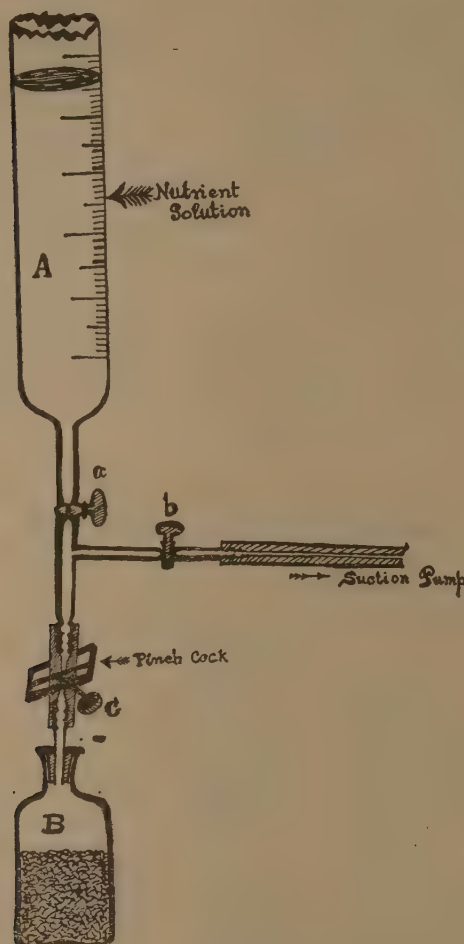


FIG. 1. Apparatus for obtaining anaerobic conditions

From time to time the mass was shaken well and in the case of aerobic and waterlogged fermentations a little fresh water was added to each bottle to replace the loss due to evaporation. It was noticed that even under waterlogged conditions there was a tendency for the materials to form a fungal growth on the surface of the water unless the mass was regularly stirred up. Under these conditions the anaerobic bacteria were no doubt mostly concerned in the decomposition in the lower part of the material, whereas the upper part in contact with the air was subjected to the action of various aerobic organisms including bacteria, fungi and actinomycetes. The waterlogged system should therefore occupy an intermediate position between aerobic and anaerobic systems.

After specified intervals the bottles were opened, the contents were filtered through a Buchner funnel and then roughly washed with distilled water. In the case of materials receiving calcium carbonate, the residue was treated

with excess of dilute hydrochloric acid (strength about 0.5 per cent) and then thoroughly washed with water immediately after the carbonate was brought into solution. It was found in a preliminary experiment that by this treatment, the pentosans and hexosans are not affected as the reaction was completed within four to five minutes. The residue was dried and aliquot portions were subjected to detailed chemical analysis (as shown in individual tables).

The experiments were conducted with different nutrient solutions (added per 20 gm. of rice straw) having the following compositions :—

1. With distilled water—
Soil solution 10 c.c.
Distilled water 190 c.c.
2. With magnesium sulphate and sodium phosphate—
Sodium phosphate solution 10 c.c. (containing 0.25 gm.).
Magnesium sulphate solution 10 c.c. (containing 0.05 gm.).
Soil solution 10 c.c.
Distilled water 170 c.c.
3. With calcium carbonate alone—
Calcium carbonate 8 gm.
Soil solution 10 c.c.
Distilled water 190 c.c.
4. With calcium carbonate and ammonium carbonate—
Calcium carbonate 5 gm.
Ammonium carbonate solution 10 c.c. (containing 0.6857 gm.)
Soil solution 10 c.c.
Distilled water 180 c.c.
5. With ammonium carbonate alone—
Ammonium carbonate solution 10 c.c. (containing 1.3714 gm.)
Soil solution 10 c.c.
Distilled water 180 c.c.
6. With sodium nitrate—
Sodium nitrate solution 10 c.c. (containing 2.4288 gm.)
Soil solution 10 c.c.
Distilled water 180 c.c.

The following experiments were performed with rice straw No. 1 and rice straw No. 2, under aerobic, anaerobic and waterlogged conditions and the tables in which the results of the analysis are shown, are mentioned below :—

Aerobic condition

Table II.—Decomposition of rice straw No. 1 with distilled water only

Table III.—Decomposition of rice straw No. 1 with magnesium sulphate and sodium phosphate

Table IV.—Decomposition of rice straw No. 1 with calcium carbonate and ammonium carbonate

Table V.—Decomposition of rice straw No. 1 with ammonium carbonate alone

Table VI.—Decomposition of rice straw No. 1 with sodium nitrate

Table VII.—Decomposition of rice straw No. 2 with distilled water only

Table VIII.—Decomposition of rice straw No. 2 with calcium carbonate and ammonium carbonate

Anaerobic condition

Table IX.—Decomposition of rice straw No. 1 with distilled water only

Table X.—Decomposition of rice straw No. 2 with distilled water only

Table XI.—Decomposition of rice straw No. 2 with sodium phosphate and magnesium sulphate

Table XII.—Decomposition of rice straw No. 2 with calcium carbonate alone

Table XIII.—Decomposition of rice straw No. 1 with calcium carbonate and ammonium carbonate

Table XIV.—Decomposition of rice straw No. 2 with calcium carbonate and ammonium carbonate

Table XV.—Decomposition of rice straw No. 2 with ammonium carbonate

Table XVI.—Decomposition of rice straw No. 2 with sodium nitrate

Waterlogged condition

Table XVII.—Decomposition of rice straw No. 1 with distilled water only

Table XVIII.—Decomposition of rice straw No. 2 with distilled water only

Table XIX.—Decomposition of rice straw No. 1 with calcium carbonate and ammonium carbonate

Table XX.—Decomposition of rice straw No. 2 with calcium carbonate and ammonium carbonate

DISCUSSION OF RESULTS OBTAINED

A study of Tables II—VIII shows that the pH of the media under aerobic conditions fell slightly during the early periods of decomposition and then gradually rose till a value slightly higher than the original was attained. This may be due to accumulation of organic acids at the early stages which, however, were completely oxidized to carbon dioxide and water with the progress of decomposition. The slight rise of pH of the media may be due to the formation of ammonia as a result of protein decomposition. In the case of Table VI, there was no fall in pH of the medium, but a gradual rise probably due to denitrification and formation of alkali carbonates even under aerobic conditions. This result is not in a line with the observations of a number of other observers who are of opinion that denitrification is not possible under ideal aerobic conditions. The difference may be due to the fact that air supply was not as complete in the depths of the medium as on the surface except for half an hour daily.

TABLE III
Decomposition of various constituents of rice straw No. I with magnesium sulphate and sodium phosphate under aerobic conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition											
		1 month		2 months		3 months		4 months		5 months		6 months	
		Total residue	Per-centage of original	Total residue	Per-centage of original	Total residue	Per-centage of original	Total residue	Per-centage of original	Total residue	Per-centage of original	Total residue	Per-centage of original
Total dry matter	87.01	51.92	59.67	45.12	51.87	39.98	45.96	35.85	40.63	31.28	35.96	27.95	32.13
Ash	13.12	6.52	...	5.82	...	5.38	...	4.69	...	4.24	...	4.12	...
Fats and waxes	1.32	0.59	44.70	0.55	41.97	0.48	36.36	0.45	34.09	0.41	31.06	0.42	31.81
Total pentosans	18.86	10.65	56.45	9.06	48.03	8.39	44.48	6.45	34.20	5.87	28.47	4.87	25.81
Crude cellulose	37.90	21.98	...	18.49	...	14.62	...	12.19	...	10.40	...	9.06	...
Ash in above	1.30	1.01	...	0.82	...	0.65	...	0.53	...	0.49	...	0.46	...
Pentosans in above	7.16	4.82	...	4.21	...	3.82	...	2.13	...	1.67	...	1.52	...
Cellulose	20.44	16.10	54.69	13.46	45.73	10.65	36.17	9.53	32.87	8.24	27.99	7.08	24.05
Crude lignin	20.68	19.23	...	18.46	...	16.48	...	16.10	...	14.73	...	13.19	...
Ash in above	6.38	5.43	...	5.40	...	4.98	...	4.28	...	3.80	...	3.81	...
Lignin	14.30	13.80	96.51	13.06	91.33	11.50	80.42	11.82	82.66	10.93	76.43	9.88	65.60
Crude protein	6.21	5.93	95.60	4.89	78.74	4.41	71.01	3.86	62.16	3.68	59.26	3.37	54.26
pH	8.05	7.77		8.15		8.20		8.47		8.59		8.59	
Per cent of protein-N ₂ in the residue	0.91	1.83		1.73		1.76		1.75		1.88		1.93	

TABLE VII

Decomposition of rice straw No. 2 with distilled water only under aerobic conditions

Chemical constituents	100 gm. original straw	Materials left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original
Total dry matter	87.55	65.75	75.11	55.08	62.92	45.28	51.72
Ash	10.15	5.62	..	5.58	..	5.27	..
Fats and waxes	0.74	0.38	51.35	0.35	47.30	0.27	36.49
Total pentosans	22.31	15.12	67.77	11.86	53.16	10.15	45.49
Crude cellulose	43.35	33.12	..	25.72	..	19.22	..
Ash in above	0.23	0.24	..	0.19	..	0.16	..
Pentosans in above	11.22	8.13	..	4.52	..	3.41	..
Cellulose	31.90	24.75	77.59	21.01	65.86	15.65	49.05
Crude lignin	22.83	19.93	..	15.90	..	13.67	..
Ash in above	5.10	3.08	..	2.93	..	2.75	..
Lignin	17.73	16.85	95.04	12.97	73.16	10.92	61.59
Crude protein	1.83	2.58	140.98	2.92	159.56	3.21	175.41
pH	6.88	6.01		6.60		6.70	
Per cent of protein-N ₂ in the residue	0.33	0.63		0.85		1.13	

TABLE VIII

Decomposition of various constituents of rice straw No. 2 with calcium carbonate and ammonium carbonate under aerobic conditions

Chemical constituents	100 gm. original straw	Materials left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original
Total dry matter	87.55	61.77	70.57	51.88	59.27	43.11	49.25
Ash	10.15	5.55	..	5.01	..	4.85	..
Fats and waxes	0.74	0.31	41.90	0.27	36.50	0.21	28.38
Total pentosans	22.31	14.63	65.58	11.28	50.56	9.54	42.76
Crude cellulose	43.35	30.84	..	23.08	..	16.95	..
Ash in above	0.23	0.23	..	0.19	..	0.17	..
Pentosans in above	11.22	7.51	..	3.95	..	2.58	..
Cellulose	31.90	23.10	72.41	18.94	59.37	14.20	44.51
Crude lignin	22.83	18.42	..	15.74	..	13.57	..
Ash in above	5.10	3.13	..	2.84	..	2.75	..
Lignin	17.73	15.29	86.24	12.90	72.76	10.72	60.46
Crude protein	1.83	2.64	144.20	3.09	168.90	3.43	187.40
pH	8.55	7.84		8.18		8.80	
Per cent of protein N in the residue	0.33	0.68		0.95		1.27	

TABLE IX

Decomposition of various constituents of rice straw No. 1 with distilled water only under anaerobic conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percentage of original	Total residue	Percentage of original	Total residue	Percentage of original
Total dry matter	87.01	60.82	69.90	54.98	63.19	50.27	57.77
Ash	13.12	6.85	..	6.12	..	5.73	..
Fats and waxes	1.32	0.89	67.42	0.81	61.36	0.75	56.82
Total pentosans	18.86	14.95	79.26	12.48	66.17	10.36	54.93
Crude cellulose	37.90	26.14	..	24.80	..	22.40	..
Ash in above	1.30	0.85	..	0.75	..	0.63	..
Pentosans in above	7.16	5.13	..	4.21	..	3.65	..
Cellulose	29.44	21.16	71.87	19.84	67.39	18.12	61.55
Crude lignin	20.68	18.92	..	17.54	..	16.17	..
Ash in above	6.38	4.64	..	3.62	..	2.59	..
Lignin	14.30	14.28	99.86	13.92	97.41	13.58	94.96
Crude protein	6.21	2.67	42.99	1.75	28.18	1.42	22.87
pH	6.88	4.91		4.40		4.20	
Per cent of protein N in the residue	0.91	0.70		0.51		0.45	

TABLE X
Decomposition of various constituents of rice straw No. 2 with distilled water only under anaerobic conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition					
		1 month		2 months		3 months	
		Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original
Total dry matter	87.55	75.98	86.78	69.95	79.91	67.77	77.41
Ash	10.15	7.41	..	5.91	..	5.82	..
Fats and waxes	0.74	0.61	82.43	0.58	78.38	0.48	64.86
Total pentosans	22.31	19.84	88.92	17.85	80.00	16.49	73.91
Crude cellulose	43.35	38.28	..	35.35	..	34.96	..
Ash in above	0.23	0.22	..	0.21	..	0.22	..
Pentosans in above	11.22	9.71	..	8.98	..	8.75	..
Cellulose	31.90	28.35	88.87	26.16	82.01	25.99	81.48
Crude lignin	22.83	22.24	..	22.15	..	22.11	..
Ash in above	5.10	5.01	..	5.20	..	5.20	..
Lignin	17.73	17.23	97.18	16.95	95.60	16.91	95.31
Crude protein	1.83	1.91	104.30	1.87	102.20	1.89	103.30
pH	6.88	5.27		4.82		4.61	
Per cent of protein N in the residue	0.33	0.40		0.43		0.45	
						4.53	
						0.46	

TABLE XIII

Decomposition of various constituents of rice straw No. 1 with calcium carbonate and ammonium carbonate under anaerobic conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original
Total dry matter	87.01	54.86	63.08	47.27	54.33	41.48	47.67
Ash	13.12	5.42	..	4.15	..	2.93	..
Fats and waxes	1.32	0.76	57.57	0.63	47.72	0.47	35.60
Total pentosans	18.86	13.64	72.31	11.42	60.54	9.52	50.47
Crude cellulose	37.90	22.85	..	20.16	..	17.04	..
Ash in above	1.30	0.84	..	0.72	..	0.51	..
Pentosans in above	7.16	4.72	..	3.82	..	2.46	..
Cellulose	29.44	17.29	58.72	15.62	53.07	14.07	47.79
Crude lignin	20.68	18.92	..	17.34	..	15.79	..
Ash in above	6.38	4.65	..	3.59	..	2.46	..
Lignin	14.30	14.27	99.79	13.75	96.15	13.33	93.24
Crude protein	6.21	1.83	29.47	1.38	22.22	1.13	18.20
pH	8.55	5.70		5.23		4.67	
Per cent of protein N in the residue	0.91	0.53		0.47		0.44	

TABLE XIV
Decomposition of various constituents of rice straw No. 2 with calcium carbonate and ammonium carbonate under anaerobic conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition											
		1 month		2 months		3 months		4 months		5 months		6 months	
		Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original	Total residue	Percent- age of original
Total dry matter	87.55	67.58	77.20	65.15	74.42	64.22	73.33	62.01	70.84	60.48	69.08	60.17	68.74
Ash	10.15	6.98	...	6.82	...	6.75	...	6.48	...	6.22	...	6.18	...
Fats and waxes	0.74	0.65	87.84	0.61	82.43	0.59	70.75	0.58	78.38	0.56	75.68	0.54	72.98
Total pentosans	22.31	17.82	79.87	16.01	71.76	15.79	70.78	15.41	69.07	15.10	67.66	14.98	67.14
Crude cellulose	43.35	34.47	...	33.85	...	32.86	...	31.49	...	30.86	...	30.80	...
Ash in above	0.23	0.21	...	0.22	...	0.20	...	0.18	...	0.19	...	0.18	...
Pentosans in above	11.22	9.72	...	9.62	...	9.12	...	8.85	...	8.69	...	8.74	...
Cellulose	31.90	24.54	76.93	24.01	75.26	23.54	73.79	22.46	70.41	21.98	68.90	21.88	68.58
Crude lignin	22.80	20.25	...	20.41	...	19.67	...	19.26	...	18.97	...	18.92	...
Ash in above	5.10	4.95	...	5.01	...	4.85	...	4.82	...	4.69	...	4.53	...
Lignin	17.73	15.30	86.30	15.40	86.86	14.82	83.60	14.64	82.58	14.38	81.12	14.39	81.17
Crude protein	1.83	1.88	102.80	1.87	102.20	1.92	104.90	1.82	99.45	1.73	94.52	1.72	93.97
pH	8.55	5.61		5.32		5.13		5.00		4.82		4.80	
Per cent of protein N in the residue	0.33	0.45		0.46		0.43		0.47		0.46		0.46	

TABLE XVII

Decomposition of various constituents of rice straw No. 1 with distilled water only under waterlogged conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percentage of original	Total residue	Percentage of original	Total residue	Percentage of original
Total dry matter	87.01	56.93	65.43	48.24	55.46	37.98	43.66
Ash	13.12	6.67	..	6.25	..	5.45	..
Fats and waxes	1.32	0.82	62.12	0.73	55.30	0.62	46.97
Total pentosans	18.86	12.83	68.01	10.68	56.61	7.92	41.99
Crude cellulose	37.90	25.33	..	19.85	..	14.99	..
Ash in above	1.30	0.83	..	0.81	..	0.55	..
Pentosans in above	7.16	4.86	..	3.79	..	2.62	..
Cellulose	29.44	19.64	66.71	14.65	49.77	11.82	40.15
Crude lignin	20.68	19.85	..	17.73	..	14.70	..
Ash in above	6.38	5.72	..	4.02	..	4.18	..
Lignin	14.30	14.13	98.81	13.71	95.87	10.52	73.57
Crude protein	6.21	3.56	57.32	2.94	47.34	2.23	35.91
pH	6.88	6.02		5.85		5.80	
Per cent of protein N in the residue	0.91	1.00		0.98		0.94	

TABLE XVIII

Decomposition of various constituents of rice straw No. 2 with distilled water only under waterlogged conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percentage of original	Total residue	Percentage of original	Total residue	Percentage of original
Total dry matter	87.55	69.11	78.93	59.34	67.78	54.02	61.72
Ash	10.15	6.23	..	5.61	..	5.25	..
Fats and waxes	0.74	0.48	64.86	0.35	47.31	0.31	41.90
Total pentosans	22.31	16.53	74.09	14.12	63.28	12.12	54.33
Crude cellulose	43.35	34.94	..	30.36	..	26.08	..
Ash in above	0.23	0.25	..	0.21	..	0.16	..
Pentosans in above	11.22	8.54	..	7.07	..	5.23	..
Cellulose	31.90	26.15	81.96	23.08	72.35	20.69	64.86
Crude lignin	22.83	21.93	..	18.30	..	17.32	..
Ash in above	5.10	4.75	..	4.82	..	4.45	..
Lignin	17.73	17.18	96.90	13.48	76.03	12.87	72.59
Crude protein	1.83	2.59	141.60	2.24	122.40	2.45	133.90
pH	6.88	6.11		5.92		5.83	
Per cent of protein N in the residue	0.33	0.60		0.60		0.73	

TABLE XIX

Decomposition of various constituents of rice straw No. 1 with calcium carbonate and ammonium carbonate under waterlogged conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percentage of original	Total residue	Percentage of original	Total residue	Percentage of original
Total dry matter	87.01	51.31	58.98	40.58	46.66	30.59	35.17
Ash	13.12	5.63	..	5.37	..	4.75	..
Fats and waxes	1.32	0.72	54.54	0.43	32.58	0.28	21.21
Total pentosans	18.86	11.38	60.34	9.45	50.09	7.01	37.16
Crude cellulose	37.90	21.36	..	16.01	..	11.82	..
Ash in above	1.30	0.82	..	0.77	..	0.58	..
Pentosans in above	7.16	4.62	..	3.15	..	1.89	..
Cellulose	29.44	16.92	57.48	12.09	41.07	9.35	31.77
Crude lignin	20.68	18.18	..	15.79	..	10.01	..
Ash in above	6.38	4.35	..	4.10	..	2.13	..
Lignin	14.30	13.83	96.74	11.69	81.75	7.91	55.33
Crude protein	6.21	3.21	51.69	2.56	41.22	1.98	31.88
pH	8.55	8.03		7.80		7.01	
Per cent of protein N in the residue	0.91	1.00		1.01		1.04	

TABLE XX

Decomposition of various constituents of rice straw No. 2 with calcium carbonate and ammonium carbonate under waterlogged conditions

Chemical constituents	100 gm. original straw	Material left after months of decomposition					
		1 month		3 months		6 months	
		Total residue	Percentage of original	Total residue	Percentage of original	Total residue	Percentage of original
Total dry matter	87.55	64.51	73.70	56.37	64.39	48.46	55.35
Ash	10.15	6.12	..	5.72	..	5.14	..
Fats and waxes	0.74	0.46	62.18	0.38	51.36	0.29	39.19
Total pentosans	22.31	16.32	73.14	13.75	61.63	11.13	49.89
Crude cellulose	43.35	32.00	..	26.35	..	20.20	..
Ash in above	0.23	0.23	..	0.20	..	0.18	..
Pentosans in above	11.22	8.12	..	5.82	..	3.43	..
Cellulose	31.90	23.65	74.16	20.33	63.71	16.59	52.00
Crude lignin	22.83	19.98	..	18.16	..	15.50	..
Ash in above	5.10	4.82	..	4.83	..	4.22	..
Lignin	17.73	15.16	85.51	13.33	75.19	12.28	69.26
Crude protein	1.83	2.47	135.00	2.99	163.40	3.27	178.60
pH	8.55	8.12		7.80		7.13	
Per cent of protein N in the residue	0.33	0.62		0.85		1.08	

As to the general course of the aerobic fermentations the results agree with the observations of most of the previous workers. A gradual decrease of fats and waxes, cellulose, hemicelluloses (pentosans), lignin, etc., was observed. The hemicelluloses disappeared more readily than cellulose during the early periods, while the order was reversed later. The mineral matter was diminished to some extent during the earlier periods but remained more or less constant during the later stages, probably due to insolubility of the residual minerals. Of all constituents the decomposition of lignins was the slowest and most incomplete.

The changes in the organic nitrogenous complexes deserve special attention. In the case of rice straw No. 1, rich in nitrogen (total nitrogen = 2.07 per cent and water-insoluble nitrogen = 0.91 per cent), in spite of decomposition there was a steady rise in the concentration of the element to a maximum of 1.7—1.9 per cent in the insoluble residue. The phenomenon appears to be a result of: (a) a gradual conversion of water-soluble nitrogenous substances of the plant material into insoluble form, (b) a relatively slower rate of disappearance of insoluble nitrogenous complexes (proteins) in comparison with that of other plant constituents at the early stages of decomposition, (c) the decomposition of the proteins at the same rate as other plant constituents, during the later stages. In the case of rice straw No. 2 poor in nitrogen (total nitrogen = 0.61 per cent and water-insoluble nitrogen = 0.33 per cent) there was no loss of the element but both a relative and an absolute increase in the concentration of the protein fraction. When no inorganic nitrogen was supplied (Table VII), the water-soluble nitrogen fraction was gradually converted into insoluble form with the progress of decomposition and when ammonium carbonate was added (Table VIII), the inorganic nitrogen was transformed into organic form (microbial protein). In the latter case, however, the synthesis of proteins by micro-organisms was considerably greater than in the former. This leads us to conclude that microbial proteins may be synthesized from either water-soluble plant nitrogen or from inorganic salts added to the medium. The simple fact that there is no decrease in the amount of proteins, as in the case of rice straw No. 2, does not, however, prove that the insoluble plant proteins are not decomposed, but probably the degradation products of the proteins are consumed, as soon as they are formed, by the micro-organisms for the synthesis of their cell-substance. This synthetic action is related to the extent of decomposition of the plant residues, being greater when the decomposition of celluloses and hemicelluloses is also greater.

As for the influence of the various nutrient solutions employed on the rate and extent of decomposition, it has been found that the decomposition of the total organic matter as a whole, as well as of the individual constituents, takes place in the following increasing order: (a) with distilled water alone (Table II), (b) with sodium phosphate and magnesium sulphate (Table III), (c) with calcium carbonate and ammonium carbonate mixture (Table IV), (d) with ammonium carbonate alone (Table V), (e) with sodium nitrate (Table VI). Similar is the case with rice straw No. 2 (Tables VII and VIII).

It is found that the addition of nitrogen salts accelerates the rate and extent of decomposition of not only rice straw No. 2 (poor in nitrogen) but also to some extent of rice straw No. 1 (rich in nitrogen). This may be due to the

fact that micro-organisms prefer inorganic nitrogen to plant proteins. Similar results were obtained by Waksman and Bavendamm [1931]. The greatest decomposition of rice straw with sodium nitrate is due to its oxidizing action and the relatively rapid decomposition of lignin in this case may be due to the resulting alkalinity of the medium in which lignin is more soluble.

However, it is found that under similar conditions, the decomposition of straw No. 2 proceeds at a much slower rate. This is due to the fact that the younger plant contains more nitrogen and water-soluble substances and is less lignified. The addition of inorganic nitrogen greatly hastens the process of fermentation of rice straw No. 2, but even then it is considerably slower than that of straw No. 1 when receiving no nitrogen salts. This is due to the more lignified character and maturity of the former.

On a careful study of Tables IX-XVI, it is found that the pH of the media progressively falls, due to the development of acidity, and the process of decomposition is abruptly slowed down as the pH value approaches about 4.6, unless neutralizing agents are used. For this purpose the use of: (1) sodium phosphate and magnesium sulphate mixture (Table XI), (2) calcium carbonate (Table XII), (3) calcium carbonate and ammonium carbonate mixture (Tables XIII and XIV) and (4) ammonium carbonate (higher dose) (Table XV) was made. The observations were similar to those made by Acharya [1935]. Ammonium carbonate (higher dose) proved most successful while calcium carbonate, though present in excess, could not retard the fall of pH of the medium. This might be due to the formation of an insoluble deposit of lime salts of the acids on the carbonate. In the case of calcium carbonate and ammonium carbonate mixture, the rate of fall of pH was slower than the former case, due to presence of ammonium carbonate, but ultimately the medium became distinctly acid, due to the insufficiency of the base.

With sodium nitrate (Table XVI), however, there was no fall of pH during the four months of decomposition but a gradual rise from 6.88 to 8.54. After this period there was again a gradual lowering to 7.88 after six months. This can be best explained as the result of the activity of denitrifying bacteria. The nitrates are reduced to atmospheric nitrogen and there is an alkalization owing to the formation of sodium carbonate. During the later stages, however, as the process of denitrification was completed, the pH of the medium gradually fell as a result of accumulation of organic acids. This idea finds support from the observations of Elma, Kluyver and Dalfsen [1934] and Acharya [1935].

The pH of the medium is the most important factor in controlling the rate and extent of decomposition in this case, as acidity greatly inhibits the reaction. The various nutrients influence the rate and extent of decomposition in the same order as in the aerobic system, the greatest decomposition being with sodium nitrate (Table XVI). However, by comparing the results of these tables with those of Tables II-VIII, it is found that in general the rate and extent of decomposition of plant materials as a whole, as well as of the various constituents, are much slower than those under aerobic conditions. The requirement of nitrogen in this case is also much lower: the amount should be only about 0.45-0.50 per cent of the total dry matter.

A study of Tables XVII-XX shows that the waterlogged system shows intermediate behaviour between aerobic and anaerobic conditions in all res-

pects. The general behaviours of the two samples of rice straw are, however, similar in all cases, e.g. straw No. 1 decomposes more readily than straw No. 2. The fall in the pH of the medium as a result of acid formation is also observed, but is not so great as under anaerobic conditions. The nitrogen requirement is also not as low as for anaerobic and not as high as for aerobic decomposition, the amount necessary in this case being about 1.00-1.08 per cent of the total dry matter. The rate and extent of decomposition is intermediate between aerobic and anaerobic conditions. This is true not only of the total organic matter as a whole, but also of the individual constituents including lignin. Acharya [1935] reported that in the case of rice straw, the greatest loss of lignin takes place under waterlogged conditions, the present investigation shows that the loss of this constituent is greatest under aerobic conditions. The influence of calcium carbonate and ammonium carbonate mixture on the rate of fermentation of rice straw under waterlogged conditions has also been found to be similar to that under the other two conditions.

SUMMARY

1. A study has been made on the decomposition of two samples of rice straw: rice straw No. 1 (younger) and rice straw No. 2 (mature) through the agency of micro-organisms present in an aqueous solution of a well-manured garden soil under aerobic, anaerobic and waterlogged conditions in presence of sufficient moisture (straw: water = 1: 10) at the ordinary laboratory temperature.

2. The younger rice straw, No. 1, was characterized by a higher content of water-soluble substances, nitrogen, ash, and lower amounts of lignin, cellulose and pentosans in comparison with the more mature rice straw, No. 2.

3. The course of decomposition was followed by measuring the losses in the amounts of the major plant constituents (cellulose, pentosans, lignin, protein, fats, waxes, etc.) which account almost completely for the loss of the total organic matter.

4. The anaerobic process of decomposition is characterized by the formation of various organic acids, combustible gases (methane, hydrogen, etc.) and other intermediate products, while under aerobic conditions, the intermediate products, if any, are quickly oxidized to carbon dioxide and water. Decomposition under waterlogged condition shows an intermediate behaviour.

5. Decomposing organic matter tends to lower the pH of the medium and inhibit the fermentation process under anaerobic conditions unless proper neutralizing agents are employed. This control of acidity is of insignificant importance under aerobic conditions where the organic matter is almost completely oxidized to carbon dioxide and water. Waterlogged systems behave intermediately.

6. Of the various media employed for control of acidity under anaerobic conditions, calcium carbonate was found to be inefficient even when present in sufficient excess, whereas calcium carbonate and ammonium carbonate mixture, though effective at the earlier stages, was ineffective during the later stages, due to the insufficiency of ammonium carbonate employed. Ammonium carbonate added to the extent of 2 per cent nitrogen on the weight of

straw (1.3714 gm. of ammonium carbonate per 20 gm. of rice straw) employed was the most successful. Sodium nitrate served a dual purpose : (a) Denitrification and rapid oxidation of organic matter, (b) Alkalization of the medium, leading to the control of acidity.

7. The process of decomposition was carried out in the following media :—

- (a) With distilled water only, (b) with sodium phosphate and magnesium sulphate mixture, (c) with calcium carbonate alone, (d) with calcium carbonate and ammonium carbonate (1 per cent nitrogen on the weight of straw), (e) with ammonium carbonate alone (2 per cent nitrogen on the weight of straw), (f) with sodium nitrate alone (2 per cent nitrogen on the weight of straw).

Other conditions remaining the same, the rate and extent of decomposition of the organic matter as a whole, as well as of the individual constituents, were found to increase when the above media were employed in the order mentioned.

8. The rate of decomposition was found to be greatest under aerobic, intermediate under waterlogged and least under anaerobic conditions, all other conditions remaining the same.

9. Under similar conditions, rice straw No. 1 decomposed much more rapidly and completely than rice straw No. 2, due to its more favourable chemical composition (*vide* paragraph 2 above). Addition of inorganic nitrogen salts greatly hastened the rate of decomposition of rice straw No. 2, and still more, the decomposition of rice straw No. 1.

10. For successful fermentation of rice straw under aerobic conditions about 1.7-1.9 per cent of nitrogen salts should be present in the medium, while under waterlogged and anaerobic conditions much less quantity (namely, about 1.00-1.08 per cent and about 0.45-0.50 per cent respectively) is sufficient. When the straw contains less than this amount of nitrogen, additional nitrogen salts are to be supplied to the medium (which are gradually converted into microbial cell substances with the progress of decomposition). If, on the other hand, the plant material contains greater than this amount of nitrogen, the excess is rapidly lost as waste products during the microbial processes, chiefly as ammonia.

11. Of the various plant constituents, the carbohydrate materials (cellulose, pentosans, etc.) are rapidly decomposed, while lignin, being resistant to decomposition, tends to accumulate with the progress of decomposition.

12. The residue left after decomposition becomes gradually poorer in carbohydrates and richer in lignins and protein-like substances and tends to approach the composition of humus.

ACKNOWLEDGEMENTS

The authors' thanks are due to Prof. J. C. Ghosh, D.Sc., F.N.I., Head of the Department of Chemistry (now Director, Indian Institute of Science,) and to Prof. S. N. Bose, Dean of the Faculty of Science, University of Dacca, for their kind interest and helpful criticisms during the progress of work.

REFERENCES

- Acharya, C. N. (1935). *Biochem. J.* **29**, 528, 953, 1116 and 1459
- Anderson, A. (1926). *Soil Sci.* **21**, 115
- Bach, M. (1926). *Landw. Vers. Sta.* **104**, 245-84
- Boruff, C. S. and Bushwell, A. M. (1929). *J. Ind. Eng. Chem.* **21**, 1181
 ————— (1930). *J. Ind. Eng. Chem.* **22**, 931
 ————— (1934). *J. Amer. Chem. Soc.* **56**, 826
- Boussingnault, J. B. (1853). *Ann. Chem. Phys.* **37**, 5-50
- Bray, M. W. and Andrews, T. M. (1924). *J. Ind. Eng. Chem.* **16**, 137-9
- Bushwell, A. M. (1930). *J. Ind. Eng. Chem.* **22**, 1168
- Deherain, P. P. (1884). *Ann. Agron.* **22**, 385-409
 ————— (1888). *Ann. Agron.* **14**, 97-133
 ————— (1902). *Traite de Agricole*. ed. 2, Paris
- Egorov, M. A. (1911). *Ann. Inst. Agron. (Moscow)* **17**, 1-59
- Elma, B., Kluyver, A. J. and Dalfsen, J. W. (1934). *Biochem. Z.* **270**, 317
- Fowler, G. J. and Joshie, G. V. (1920). *J. Ind. Inst. Sci.* **3**, 39
- Hebert, A. (1892). *Ann. Agron.* **18**, 536
- Hebert, A. and Heim, F. (1911). *Ann. Sci. Agric.* (3) **6** (II), 337-47
- Hoppe-Seylor, F. (1899). *Ztschr. Physiol. Chem.* **13**, 66-121
- Hunt, T. F. (1889). *III. Agric. Expt. Sta. Bull.* **5**, 141-64
- Konig, J. (1926). *Mitt. Deuts. Landw. Gessel. Stuck.* **26** & **27**
- Langwell, H. and Lymn, H. (1923). *J. Soc. Chem. Ind.* (T) **42**, 280
 ————— (1932). *J. Soc. Chem. Ind.* (R) **51**, 988
- Lathorp, E. C. (1912, 1). *8th Internat. Congr. Appl. Chem.* **15**, 147-51
 ————— (1912, 2). *J. Chem. Soc.* **34**, 1260-3
 ————— (1917, 1). *J. Frankl. Inst.* **183**, 169-206, 303-21, 465-98
 ————— (1917, 2). *J. Ind. Eng. Chem.* **3**, 657-67
- Norman, A. G. and Jenkins, S. H. (1933). *Biochem. J.* **27**, 818-31
- Omeliansky, W. (1895). *Compt. Rend.* **121**, 653-5
 ————— (1897). *Compt. Rend.* **125**, 970-5, 1131-5
- Ost, H. and Wilkening, L. (1910). *Chem. Zeit.* **34**, 461-62
- Phillips, M., Weihe, H. D. and Smith, N. R. (1930). *Soil Sci.* **30**, 383-90
- Rege, R. D. (1927). *Ann. Appl. Biol.* **14**, 1-44
- Richards and Amooore (1920). Unpublished data.
- Richards, E. H. and Norman, A. G. (1927). *Biochem. J.* **25**, 1769
- Rose, R. E. and Lisse, M. W. (1917). *J. Ind. Eng. Chem.* **9**, 284-7
- Schwalbe, C. G. (1925). *Papier Faber.* **23**, 174
- Waksman, S. A. (1926, 1). *Proc. Internat. Soc. Soil Sci.* **2**, 293-304
 ————— (1926, 2). *Soil Sci.* **22**, 123-62, 221-32, 323-33, 395-406, 421-36
 ————— (1927). *Cellulose Chem.* **8**, 97-103
- Waksman, S. A. and Bavendamm, W. (1931). *J. Bact.* **22**, 91-102
- Waksman, S. A. and Diehm, R. A. (1931). *Soil Sci.* **22**, 97-117, 119-39
- Waksman, S. A. and Gerretsen, F. C. (1931). *Ecology* **12**, 33-60
- Waksman, S. A. and Heukelekian, H. (1925). *J. Biol. Chem.* **66**, 323-42

- Waksman, S. A. and Hutchings, I. J. (1936). *Soil Sci.* **42**, 119-30
- Waksman, S. A. and Skinner, C. E. (1926). *J. Bact.* **12**, 57-84
- Waksman, S. A. and Tenney, F. G. (1926). *Soil Sci.* **22**, 395
- (1927). *Soil Sci.* **24**, 275-83, 317-34
- (1928). *Soil Sci.* **26**, 155-71
- (1929). *Soil Sci.* **28**, 55-84. 315-40
- (1930). *Soil Sci.* **30**, 143-60
- Waksman, S. A., Tenney, F. G. and Stevens, K. R. (1928). *Ecology* **9**, 126-44

II. A NOTE ON THE CHANGES IN THE METHOXYL AND NITROGEN CONTENT OF LIGNIN OF RICE STRAW DURING ITS DECOMPOSITION BY MICRO-ORGANISMS

BY

S. S. GUHA SIRCAR

S. C. DE

AND

H. D. BHOWMICK

Department of Chemistry, University of Dacca, Dacca

(Received for publication on 28 December 1938)

RECENT researches have supported the prevalent idea that lignin, the resistant constituent of plants, takes the most prominent part in the formation of humus, during their micro-biological decomposition. In part I of the present investigation also, it has been found that plant residues become gradually richer and richer in lignin with the progress of decomposition as the latter breaks down only slowly in comparison with other plant constituents. However, the decomposition of this constituent has been found to be the greatest under aerobic, intermediate under waterlogged and the least under anaerobic conditions. Fuchs [1926] summarized the experimental evidences of various investigators which tended to establish the role of lignins as the mother substances of humus or of humic acids. He stated that the transformation is accompanied by the splitting off of methoxyl groups, the formation of phenolic, hydroxyl and carboxyl groups and self-condensations. Various other investigators, such as Hoppe-Seylor [1899], Page [1932] and Waksman and others [1932-34] have also held that humic acid is formed by the combination of lignin or modified lignin complexes with proteins during the micro-biological decomposition of plant residues. Hebert [1892, 1893] and Deherain [1902] were the first to suggest the conception of humus as a mixture of lignin and protein, the former being the resistant part of the plant residues and the latter synthesized by micro-organisms inhabiting them. According to Hobson and Page [1932] the nitrogen in humus is associated with humic matter in some manner which does not allow of its removal by methods which ordinarily remove physically bound nitrogen compounds. A series of ligno-protein complexes were prepared by Waksman and Iyer [1932-33] which behaved in most respects, such as colour, solubility in alkalis, chemical reactivity and resistance to attack by micro-organisms like the typical humic acids, humic matter or alphafraction of humus.

Waksman and Smith [1934] have observed the gradual removal of methoxyl groups in lignin in the process of natural decomposition of organic residues under aerobic but more specially anaerobic conditions, but an increase in the relative amount of ash and organic nitrogenous compounds. The lignin molecule is modified considerably during decomposition even when it is not destroyed as a whole. Under aerobic conditions, however, the lignin molecule as a whole is attacked, and the methoxyl content of the residual lignin is not modified to any marked extent.

An attempt has been made in this paper to follow the changes undergone by the lignin fraction during the progress of micro-biological decomposition. For this purpose the lignins obtained from rice straw decomposed under various conditions of the medium under aerobic, anaerobic and waterlogged conditions (as described in part I) were analysed for: (i) methoxyl content, and (ii) nitrogen.

Now the methoxyl, which is a characteristic group of lignin, is not present in constant proportion in various preparations, but its amount varies from source to source of the lignin as was shown by Fuchs [1926], and even in the same plant at different stages of growth as was shown by Beckmann [1923]. Beckmann also found that not only is there an increase in the lignin content of plants with an increase in maturity, but the amount of methoxyl also increases.

EXPERIMENTAL

The lignin preparations used for the present study were all obtained from rice straws No. 1 and No. 2, as described in part I, at various stages of decomposition and the same methods were employed for their isolation to obtain comparative results. In part I the method of isolation of lignin has been described. The sulphuric acid method has two advantages: (i) The lignin is isolated quantitatively and free from carbohydrates, and (ii) it contains all the methoxyl groups intact.

It has been recently shown by Harris, Sherrard and Mitchel [1934] that fuming hydrochloric acid method of isolation of lignin as recommended by Willstatter and Zechmeister [1931] is open to objection because part of the methoxyl is lost during this treatment.

The methoxyl values were determined by Zeisel's method as modified by Perkin [1903]. The nitrogen was estimated by micro-Kjeldahl method as only small quantities of samples were available. The distillation apparatus is described in Pregl's [1924] '*Quantitative micro analysis*' (translated by Fyleman). The results of analysis calculated per 100 gm. of lignin employed are expressed in Tables I-IV.

TABLE I

Changes in the methoxyl content of lignin of rice straw No. I with decomposition under aerobic, anaerobic and waterlogged conditions

Treatment	Original lignin (per cent)	After 3 months (per cent)	After 6 months (per cent)
Aerobic with distilled water only	8.47	7.17	6.87
Aerobic, with CaCO_3 and $(\text{NH}_4)_2\text{CO}_3$..	7.78	6.34
Waterlogged, with distilled water only	..	5.60	4.89
Waterlogged, with CaCO_3 and $(\text{NH}_4)_2\text{CO}_3$..	5.82	5.31
Anaerobic, with distilled water only	8.47	4.69	3.85
Anaerobic, with CaCO_3 and $(\text{NH}_4)_2\text{CO}_3$..	4.75	4.01

TABLE II

Changes in the methoxyl content of lignin of rice straw No. 2 with decomposition under aerobic, anaerobic and waterlogged conditions

Treatment	Original lignin (per cent)	After 3 months (per cent)	After 6 months (per cent)
Aerobic, with distilled water only	9.42	7.25	6.62
Aerobic, with CaCO_3 and $(\text{NH}_4)_2 \text{CO}_3$..	8.47	7.82
Waterlogged, with distilled water only	..	6.49	5.63
Waterlogged, with CaCO_3 and $(\text{NH}_4)_2 \text{CO}_3$..	6.52	5.87
Anaerobic, with distilled water only	..	5.59	4.89
Anaerobic, with CaCO_3 and $(\text{NH}_4)_2 \text{CO}_3$..	6.01	5.65

TABLE III

Changes in the nitrogen content of lignin of rice straw No. 1 with decomposition under aerobic, anaerobic and waterlogged conditions

Treatment	Original lignin (per cent)	After 3 months (per cent)	After 6 months (per cent)
Anaerobic, with distilled water only	1.25	1.28	1.51
Anaerobic, with CaCO_3 and $(\text{NH}_4)_2 \text{CO}_3$..	1.31	1.63
Waterlogged, with distilled water only	..	1.30	1.72
Waterlogged, with CaCO_3 and $(\text{NH}_4)_2 \text{CO}_3$..	1.39	1.87
Aerobic, with distilled water only	..	1.50	2.37
Aerobic, with CaCO_3 and $(\text{NH}_4)_2 \text{CO}_3$..	1.68	2.58

TABLE IV

Changes in the nitrogen of lignin of rice straw No. 2 with decomposition under aerobic, anaerobic and waterlogged conditions

Treatment	Original lignin (per cent)	After 3 months (per cent)	After 6 months (per cent)
Anaerobic, with distilled water only	1.11	1.13	1.36
Anaerobic, with CaCO ₃ and (NH ₄) ₂ CO ₃	..	1.28	1.49
Waterlogged, with distilled water only	..	1.30	2.15
Waterlogged, with CaCO ₃ and (NH ₄) ₂ CO ₃	..	1.38	2.48
Aerobic, with distilled water only	..	1.67	2.85
Aerobic, with CaCO ₃ and (NH ₄) ₂ CO ₃	..	1.73	2.98

DISCUSSION OF RESULTS

A study of Tables I-IV shows that the methoxyl content of lignin of rice straw No. 2 (mature) is higher than that of lignin of straw No. 1 (less mature), but the nitrogen content is lower. It has been found that the presence of nitrogen in lignin of original plant materials cannot be eliminated even after repeated treatments with sulphuric acid. This may be due to two reasons: (1) The nitrogen may be an integral part of the molecule, (2) Under the influence of concentrated acid the proteins may be rendered partly insoluble (humins).

Lignin from straw No. 1 contains more nitrogen than that from No. 2. This may be due to a greater amount of protein being precipitated by the mineral acids if the second assumption is correct.

A glance at Tables I and II will show that with the progress of decomposition there is a decided loss of the methoxyl content of lignin under aerobic and anaerobic as well as under waterlogged conditions, the loss being greatest under anaerobic, intermediate under waterlogged, and least under aerobic conditions. It is also found that the loss of methoxyl is not so great when the medium contained a mixture of calcium carbonate and ammonium carbonate than when the straw decomposed alone. Possibly the acidity of the medium helps the splitting off of the methoxyl group.

These facts are supported by the observations of Waksman and Smith [1934] who found that aerobic organisms attack the lignin molecule as a whole and completely destroy it without affecting the methoxyl content of the material. Under anaerobic conditions, however, the decomposition of lignin is the least but the methoxyl group is rapidly split off. The waterlogged system shows an intermediate behaviour.

On the other hand, a study of Tables III and IV shows that nitrogen content of lignin increased with increasing periods of fermentation, the increase being most marked under aerobic, intermediate under waterlogged and least under anaerobic conditions. This may probably be due to: (i) the greater activity of aerobic organisms resulting in the synthesis of greater amounts of microbial proteins, or (ii) the greater activity of aerobic organisms in the synthesis of ligno-proteins.

It is also to be noted that the addition of nitrogen as ammonium carbonate in the decomposing rice straw also increases the formation of ligno-protein complexes.

Attempts were made to measure the rate of formation of humic acids (the alkali-soluble and acid-insoluble fraction of the decomposing organic matter) with the progress of decomposition of rice straw. But as both the original lignin and lignin-humic acid mixture of the decomposed straw were somewhat soluble in alkalies, these attempts did not throw much light on the problem. Karrer and Bodring-Wieger [1921, 1923] demonstrated that when acetyl bromide is allowed to act upon plant material, practically all of the organic constituents, except 'humified matter', are brought into solution, forming acetylated and methylated products; and he used this method for the separation of the undecomposed plant residues from the 'humified' substances. But it was found that this action of acetyl bromide is extremely slow and at least four to five days contact with the hot reagent was necessary before much effect was produced.

The action of this reagent and also the conductometric titrations of humified lignins are under investigation.

SUMMARY

1. A study has been made of the changes in the content of nitrogen and methoxyl in the lignin preparations (obtained from straw No. 1 and straw No. 2) at various stages of decomposition under aerobic, anaerobic and waterlogged conditions

2. There is an increase in the nitrogen and decrease in the methoxyl content of lignin with the progress of decomposition. When the plant materials are decomposed with a mixture of calcium carbonate and ammonium carbonate, the loss of methoxyl is less but the nitrogen content is higher.

3. Loss of methoxyl is the greatest under anaerobic, intermediate under waterlogged, and the least under aerobic conditions. The reverse is the case with the increment of nitrogen content.

ACKNOWLEDGMENTS

The authors' thanks are due to Prof. J. C. Ghosh, D.Sc., F.N.I., Head of the Department of Chemistry (now Director, Indian Institute of Science, Bangalore) and to Prof. S. N. Bose, Dean of the Faculty of Science, University of Dacca, for their kind interest and helpful criticisms during the progress of work.

REFERENCES

- Beckmann, E., Liesche, O. and Lehmann, F. (1923). *Biochem. Ztschr.* **139**, 491-508
- Deherain, P. P. (1902). *Traité de Chimie Agricole*, ed. 2, Paris
- Fuchs, W. (1926). *Die Chemie des Lignins*, Berlin
- Harris, E. E., Sherrard, E. C. and Mitchel, R. L. (1934). *J. Amer. Chem. Soc.* **56**, 889-93
- Herbert, A. (1892, 1). *Ann. Agron.* **18**, 536-50
- (1892, 2). *Compt. Rend. Acad. Sci.* **115**, 1321-3
- (1893). *Expt. Sta. Record* **5**, 141-58
- Hobson, R. P. and Page, H. J. (1932). *J. Agric. Sci.* **22**, 497-515
- Hoppe-Seylor, F. (1899). *Ztschr. Physiol. Chem.* **13**, 66-121
- Karrer, P. and Boding-Wieger, B. (1921). *Helv. Chim. Acta.* **4**, 700
- (1923). *Helv. Chim. Acta.* **6**, 817-22
- Page, H. J. (1932). *J. Agric. Sci.* **22**, 291-6
- Perk in, W. H. (1903). *Proc. Chem. Soc.* **19**, 1370
- Pregl, F. (1924). *Quantitative Micro-analysis*. (Translated by E. Fyleman). London, J. & A. Churchil. Page 96
- Waksman, S. A. and Iyer, K. R. N. (1932, 1). *J. Wash. Acad. Sci.* **22**, 41-50
- (1932, 2). *Soil Sci.* **34**, 43-80
- (1933). *Soil Sci.* **36**, 57-82
- Waksman, S. A. and Smith, H. W. (1934). *J. Amer. Chem. Soc.* **56**, 1225
- Willstatter, R. and Zehmmeister, L. (1931). *Ind. Eng. Chem. Anal. Ed.* **3**, 253

STUDIES ON THE CHEMICAL CONSTITUENTS OF INDIAN LATERITIC AND RED SOILS

I. DETERMINATION OF FREE SESQUIOXIDE COMPONENTS

BY

S. P. RAYCHAUDHURI

AND

M. SULAIMAN

Agricultural Chemistry Section, Dacca University

(Received for publication on 28 September 1939)

Introduction

THE determination of free alumina and free iron oxide is important for the proper characterization of red soils. Bauer's [1898] work showing that the laterites of Seychilles was similar to the so-called bauxite of Hessa in Germany not merely drew attention to the fundamental chemical character of laterite, but left little else than details to be done by others on the nature of laterites. Dealing with some aspects of tropical soils, Hardy [1935] writes : ' Evidently the present usage of the term laterite by petrologists is inexact, and the modern definition has come to mean a highly aluminous and highly hydrated residual rock product, usually also rich in hydrous iron oxides and containing other characteristic components '. It has been used in this sense by Harrison [1933], by many other early soil investigators and geologists. Mention may also be made here of the views of Warth and Warth [1903], who, as a result of their detailed chemical examination of many typical Indian laterites, write : ' Further, the results show the term laterites has a distinct meaning throughout the many varieties of this rock. Laterite is bauxite in various degrees of purity from the richest wochenite down to such specimens, in which the free alumina has entirely disappeared '. The determination of free alumina and free iron oxides at different horizons of the red soil profiles of India was accordingly undertaken.

Different methods have been suggested by different workers for the determination of the sesquioxide components, viz. by Van Bemmelen [1933], Tamm [1922], Mattson [1931], Hardy [1931], Drosdoff and Truog [1935], and recently by Truog and co-workers [1937]. Tamm's ammonium oxalate method seems to be very drastic treatment and of the other methods the one most commonly used is that devised by Hardy. Recent investigations however indicate that methods of Drosdoff and Truog (which has been later modified by Truog and co-workers), is most convenient for the determination of free iron oxide components of soils. Accordingly, as a preliminary stage of this work, the data of the percentages of sesquioxide components of soils were obtained following the methods of Hardy and of Drosdoff and Truog.

Experimental

I. DETERMINATIONS BASED ON THE PRINCIPLE OF ALIZARIN ADSORPTION USED BY HARDY [1931]

In this procedure the amount of alumina and iron oxide uncombined with silica is determined by the adsorption of alizarin, the determinations being based on the fact that the iron oxide in the soil can adsorb alizarin sulphonate only before ignition, whilst the alumina gel can adsorb alizarin sulphonate only after ignition.

Two portions were taken, each of mass one gram (70 I.M.M.). One portion was heated to dull redness (800°C.) for six minutes in a silica crucible covered by a lid, the other was not heated. Each portion was introduced into a Pyrex glass test tube containing 20 c.c. of 0.5 per cent solution of sodium alizarin sulphonate (alizarin—S)*. Adhering particles were washed down the sides of the tubes with 10 c.c. of 80 per cent boric alcohol. The tubes were heated with simple condensers and then heated in a gently boiling water bath for ten minutes. After settling for five minutes, the supernatant liquid in each tube was decanted into a Buchner funnel containing filter paper pulp, and filtered by suction into a filtering flask. The solid material was treated with 25 c.c. boric alcohol, boiled, and the whole suspension poured into the funnel and filtered by suction. Excess of dyestuff was washed out of the sediment with a little boiling boric alcohol followed by boiling distilled water.

The adsorbed dyestuff from the stained material was extracted by means of a saturated aqueous solution of sodium oxalate containing sufficient free oxalic acid to impart a pH value of 3.8. The concentration of the dyestuff in the extract was measured in a Duboseq colorimeter against a standard. From the results both the alumina content and the ironoxide content of the soils could be calculated.

A blank oxalate extraction on 1 gm. of the material, both fresh and ignited, omitting the dyestuff treatment, was performed in all cases to correct for the iron solubility of ferruginous materials.

II. DETERMINATIONS BASED MAINLY ON THE PRINCIPLES DEVISED BY DROSDOFF AND TRUOG [1935]

(A) *Separation and determination of free silica and free alumina*

Two gm. of soil (70 I.M.M.) were digested with 2 per cent sodium carbonate solution in a 250 c.c. Pyrex beaker at about 70°C. for 10 hours with frequent stirring by glass rod to dissolve free silica and free alumina. The content of the beaker was decanted and washed several times with 0.05 N hydrochloric acid, using an Ecco centrifuge. The washings were analysed for free silica and free alumina.

(B) *Determination of free iron oxide*

The residue containing soil from (A) was suspended in 250 c.c. water in a 500-c.c. bottle and saturated with sulphuretted hydrogen for half an hour. It was then made just alkaline with normal ammonium hydroxide, shaken for half an hour, acidified with decinormal hydrochloric acid, adding an excess

* 100 c.c. of the solution contained about 80 c.c. of 80 per cent alcohol saturated with boric acid (pH 3.2).

of about 50 c.c. to dissolve the iron sulphides completely, and then warmed on water bath to drive off the sulphuretted hydrogen and coagulate the suspension which was then transferred to the centrifuge tubes and the supernatant liquid was collected by decanting after centrifuging. The residue was washed several times with 0.05 *N* hydrochloric acid by centrifuging. The supernatant liquid and washings were collected and the quantity of iron oxide was determined in the solution. Soils containing large amounts of free iron oxide may require more than one treatment for complete removal of iron oxide.

Results

Table I shows the results of the determination of free alumina and free iron oxide by Hardy's method with some typically Indian red soil samples on profile basis, whilst Table II gives the comparison of the data of free sesquioxides obtained by the methods of Hardy and of Drosdoff and Truog. The percentages of free silica obtained by the latter method is also included in the same Table II. The figures for the percentages of free alumina have been calculated by the new calculating factor (0.1) given by Hardy [Hardy and Rodrigues, 1939].

TABLE I
Percentages of free sesquioxide components obtained by the Hardy's method

Soil No.	Locality	Depth	Oven dry basis		
			Per cent free Al_2O_3	Per cent free Fe_2O_3	Per cent total free $\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$
1p	Dacca, Bengal . .	0 in.—6 in. .	1.02	2.14	3.16
2p	Dacca, Bengal . .	6 in.—2 ft. 3 in.	1.80	3.89	5.69
3p	Dacca, Bengal . .	2 ft. 3 in.—4 ft.	2.40	4.90	7.30
4p	Suri, Bengal . .	0 ft.—1 ft. .	1.56	2.22	3.78
5p	Suri, Bengal . .	1 ft.—1 ft. 6 in.	1.81	2.35	4.16
6p	Suri, Bengal . .	1 ft. 6 in.—4 ft.	2.08	3.33	5.41
7p	Suri, Bengal . .	Below 13 ft. .	1.98	1.05	3.03
8p	Suri, Bengal . .	Below 13 ft..	1.08	0.93	2.01
10p	Bidar, Hyderabad .	0 ft.—1 ft. .	6.62	2.68	9.30
13p	Bidar, Hyderabad .	0 in.—1 ft. 6 in.	5.92	3.00	8.92
14p	Didgi, Hyderabad .	Surface layer	2.50	4.77	7.27
15p	Didgi Hyderabad .	51 ft.—54 ft.	7.06	5.12	12.18
16p	Jairabad, Hyderabad .	0 in.—1 ft. 6 in.	4.98	2.68	7.66
18p	Himayetsagar, Hydera- bad	0 in.—3 in. .	0.91	1.67	2.58
19p	Himayetsagar, Hydera- bad	3 in.—1 ft. 6 in.	0.72	3.78	4.50
20p	Himayetsagar, Hydera- bad	1 ft. 6 in.—4 ft.	1.40	1.05	2.45

TABLE I—*contd.*

Soil No.	Locality	Depth	Oven dry basis		
			Per cent free Al_2O_3	Per cent free Fe_2O_3	Per cent total free $\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$
21p	Himayetsagar, Hyderabad	1 ft. 6 in.—4 ft.	3.04	2.09	5.13
23p	Telankheri, Nagpur, C. P.	0 in.—2 in. .	10.30	3.92	14.22
24p	Telankheri, Nagpur, C. P.	2 in.—2 ft. 6 in.	8.38	6.85	15.23
26p	Telankheri, Nagpur, C. P.	13 ft.—16 ft..	1.08	1.04	2.12
27p	Telankheri, Nagpur, C. P.	16 ft.—21 ft..	0.54	2.47	3.01
33p	Raipur, C. P. . .	0 in.—4 in. .	6.33	3.47	9.80
34p	Raipur, C. P. . .	4 in.—1 ft. 5 in.	6.74	3.97	10.71
35p	Raipur, C. P. . .	1 ft. 5 in.—4 ft.	7.62	4.68	12.30
36p	Raipur, C. P. . .	0 in.—6 in. .	5.82	2.10	7.92
37p	Raipur, C. P. . .	0 in.—6 in. .	4.09	2.53	6.62
38p	Labhandi, Raipur, C. P.	0 in.—8 in. .	4.38	2.51	6.89
39p	Labhandi, Raipur, C. P.	8 ft.—10 ft. .	3.32	1.55	4.87
42p	Alisagar, Hyderabad .	0 ft.—1 ft. .	3.24	4.37	7.61
43p	Alisagar, Hyderabad .	1 ft. and down	5.14	3.78	8.92
53p	Nilgiri Hills } (1)—3000 ft.	0 in.—1 ft. 8 in.	1.86	3.25	5.11
54p	Nilgiri Hills }	1 ft. 8 in.—3 ft.	1.94	4.40	6.34
55p	Nilgiri Hills } a.s.l.	Below 54p .	1.44	3.78	5.22
56p	Nilgiri Hills } (2)—5000 ft.	0 ft.—1 ft. .	9.53	7.19	16.72
57p	Nilgiri Hills }	1 ft.—2 ft. .	9.62	7.37	16.99
58p	Nilgiri Hills } a.s.l.	2 ft.—6 ft. .	11.64	6.90	17.54
59p	Nilgiri Hills } (3)—7000 ft.	0 in.—1 ft. .	13.70	7.99	21.69
60p	Nilgiri Hills }	1 ft.—3 ft. .	20.84	10.00	30.84
61p	Nilgiri Hills } a.s.l.	3 ft.—4 ft. 6 in.	17.34	7.18	24.52
62p	Nilgiri Hills }	4 ft. 6 in.—6 ft.	17.01	7.07	24.08
63p	Guntur, Madras . .	0 in.—9 in. .	9.04	6.23	15.27
64p	Guntur, Madras . .	9 in.—(5 ft.—6 ft.)	2.96	4.95	7.91

TABLE II

Comparison of the data of the free sesquioxide obtained by the methods of Hardy and of Drosdoff and Truog

Soil No.	Oven dry basis				
	Hardy's method		Drosdoff and Truog's method		
	Per cent free Al_2O_3	Per cent free Fe_2O_3	Per cent free SiO_2	Per cent free Al_2O_3	Per cent free Fe_2O_3
33p . .	6.33	3.47	0.0919	0.495	6.01
34p . .	6.74	3.97	0.1038	0.757	6.42
35p . .	7.62	4.68	0.1349	0.497	7.37
53p . .	1.86	3.25	0.1197	0.522	4.74
54p . .	1.94	4.40	0.1715	0.764	6.70
55p . .	1.44	3.78	0.1503	0.477	5.70
56p . .	9.53	7.10	0.1826	0.522	7.78
57p . .	9.62	7.37	0.1954	1.065	8.21
58p . .	11.64	6.90	0.2174	1.007	7.42
59p . .	13.70	7.99	0.1847	0.819	11.51
60p . .	20.84	10.00	0.1086	0.517	15.47
61p . .	17.34	7.18	0.1193	0.363	11.67
62p . .	17.01	7.07	0.1614	0.396	10.22

Discussion

It will be seen from Table I that the percentages free iron oxides in the soils bear no relation to the percentages of free alumina. It is also found that in the case of the Dacca profile the contents of both alumina and of iron oxide increase down the profile. In the case of the profile from the Suri, the percentages of both oxides show a maximum at an intermediate depth. The profile from Himayetsagar shows a minimum percentage of free alumina at intermediate depth, whilst the percentage of free iron oxide shows a maximum at an intermediate depth. In the case of Telankheri profile at Nagpur, the percentage of free alumina decreases down the profile, whilst the percentage of free iron oxide shows a maximum at an intermediate depth. In the case of the Raipur profile in the Central Provinces both the percentages of alumina and iron oxide increase as the depth of the profile increases. The profiles from the Nilgiri Hills, in general, show a maximum concentration of alumina and iron oxide at an intermediate depth of the profile.

Except in the case of soils from Nagpur and Nilgiri Hills (2) and (3), and the top layer of Guntur soils the percentage of free alumina in the soil samples is never very high, so that, judged from the point of view of Bauer

all the so-called-lateritic soils of India in Table I, cannot be classed as laterites or lateritic. This conclusion also appears to be evident from a consideration of the $\text{SiO}_2/\text{Al}_2\text{O}_3$ and $\text{SiO}_2/\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$ ratios of the clay fractions*. The $\text{SiO}_2/\text{Al}_2\text{O}_3$ ratios of clay fractions are often greater than 2 which suggest that the soils cannot be classed as laterites or lateritic in the sense of the definition by Martin and Doyné [1930], although they are known to be such by the departments of Agriculture of the respective provinces from where the soils were collected.

The data in Table II indicate that the percentages of the free alumina obtained by Hardy's method are much higher than those obtained by the method of Drosdoff and Truog. On the other hand, the percentages of iron oxides obtained by Hardy's method are somewhat smaller. There appears to be no parallelism between the results obtained by the two methods. In view of the considerations set forth above, it is felt desirable to examine more closely the validity and usefulness of different methods for estimating the free sesquioxide components in Indian lateritic soils. Such investigations are in progress.

Thanks of the authors are due to Prof. J. C. Ghosh for his kind interest during the progress of this work and to the Imperial Council of Agricultural Research by whose grant the soil samples used in the present investigation were collected. Thanks are also due to the Bengal Government for the award, to one of the authors (M. S.), of a research scholarship which has enabled him to carry out this work.

Summary

1. The percentages of free sesquioxides in Indian lateritic and red soils have been determined on profile basis following the methods devised by Hardy and by Drosdoff and Truog.
2. The percentages of free iron oxides obtained by Hardy's method are somewhat smaller than those obtained by the method of Drosdoff and Truog.
3. The percentages of free alumina obtained by Hardy's procedure are, on the other hand, much higher than those obtained by the other method.
4. There is no correlation between the results obtained by the two methods.

REFERENCES

- Bauer, M. (1898) *Neuss Jahrb. F. Min. Geol. U. Pal.* **2**, 163-219
 Drosdoff, M. and Truog, E. (1935) *J. Amer. Soc. Agron.* **27**, 312-17
 Hardy, F. (1931) *J. Agric. Sci.* **21**, 150-66
 Hardy, F. (1935). *Trans. 3rd. Internat. Cong. Soil Sci.* **2**, 153
 Hardy, F. and Rodrigues, G. (1939). *Soil. Sci.* **48**, 361-84,
 Harrison, J. B. (1933). *The Katamorphism of igneous rocks under humid tropical conditions*. Imp. Bur. Soil. Sci., Rothamsted Exptl. Sta., Harpenden, England
 Martin, F. J. and Doyné, H. C. (1930). *J. Agric. Sci.* **20**, 135
 Mattson, S. (1931). *Soil Sci.* **31**, 311-31
 Tamm, O. (1922). *Medl. Statens Skogforsokenst.* **19**, 387-404
 Truog, E., et al. (1937). *Proc. Soil Sci. Soc. Amer.* **1936**, **1**, 101-12
 Van Bemmelen, J. M. (1933) (cp. W. Bujakowsky and C. Treschow, k Ø benhavn, (1933), 121-41)
 Warth, H. and Warth, F. J. (1903). *Geol. Mag.* **4**, 155

* Unpublished work.

THE DEPTH OF THE SURFACE LAYER OF THE SOIL TAKING PART IN THE DIURNAL EXCHANGE OF MOISTURE WITH THE AIR LAYERS NEAR THE GROUND

BY

A. K. MALLIK, M.Sc., B.Sc. (Ag.), Assoc. I.A.R.I.
Assistant Agricultural Meteorologist, Meteorological Office, Poona
(Received for publication on 15 August 1939)

INTRODUCTION

IT has been shown in a series of papers [Katti, 1935 ; Ramdas, 1934 ; Ramdas *et al.*, 1934, 1935, 1936, 1938, 1939] that during the clear season at Poona, when the 'surface layer' of the soil is so dry as to contain hygroscopic moisture only, the soil loses moisture by evaporation into the atmosphere from morning till afternoon but from the afternoon till the next morning it absorbs from the atmosphere more or less all the moisture lost during the earlier part of the day. Thus there is a regular sequence of maximum and minimum moisture content epochs of the soil at about the minimum and the maximum temperature epochs respectively. The exchange of moisture is greatest in the black cotton soil, much less in the alluvial soil and practically absent in quartz powder. The exchange of moisture is confined to the surface layer of the soil but the exact thickness of the layer involved in the diurnal exchange of moisture remained to be found out. This problem received attention during the clear season of 1939. The clear season at Poona is characterized by cloudless skies, a large diurnal range of temperature and of relative humidity and feeble air movements. During the period March to June 1939 some experiments were made to find out the exact thickness of the 'surface layer' of the soil exchanging moisture with the air layers near the ground. A short note on the subject describing only the results in the case of the black cotton soil of Poona was published recently. In the present paper the results obtained with some other typical Indian soils are discussed in detail.

MATERIALS AND METHODS

A series of cylindrical brass vessels 4.75 cm. in diameter were made with their tops open and the bottoms closed. The series of cylinders were made with increasing heights for exposing soils with depths ranging from 1 to 40 mm. The soils under study, thoroughly air-dried and passed through a 1 mm. sieve, were filled in these pots, the actual depths of soil being 1, 2, 3, 4, 5, 10, 20 and 40 mm. respectively. These vessels were kept embedded in the ground with their tops fully exposed. It was arranged that the surface of the soil in each experimental vessel was at the same level as that of the

soil outside. On selected clear days these vessels were exposed in the open and weighed at intervals to find out the maximum and minimum weights of the soil due to the gain or loss of moisture in the process of exchange with the atmosphere. The following soils were included in the study :—

- (i) Black cotton soil of Poona.
- (ii) Red soil of Bangalore.
- (iii) Alluvial soil of Lyallpur.
- (iv) Sandy soil of Trivandrum.

STATEMENT OF RESULTS

(a) *Exchange of moisture by the black cotton soil of Poona as shown by the maximum and minimum weights*

Poona soil was filled in the vessels ranging from 1 to 40 mm. in depth and these were exposed to the open on a series of clear days in March, April and May 1939. The maximum and minimum weights were obtained by weighing these vessels with the soil at 6 A.M. in the morning and at 2 P.M. in the afternoon respectively. Tables I (a), (b), (c) and (d) give the difference between the maximum and minimum weights for various depths of soil on the different occasions.

TABLE I(a)

Black cotton soil of Poona (maximum-minimum weight in gm.)

Depth of soil (mm.)	23-3-1939	24-3-1939	25-3-1939	26-3-1939	Mean
1	0.1945	0.1952	0.1902	0.1918	0.1929
2	0.3532	0.3612	0.3610	0.3808	0.3640
3	0.4640	0.5016	0.5248	0.4986	0.4972
4	0.5818	0.5688	0.5544	0.6140	0.5798
5	0.6918	0.6656	0.6304	0.6532	0.6600
10	0.7808	0.7708	0.7634	0.7732	0.7720
20	0.7402	0.7418	0.7576	0.7104	0.7373
40	0.7216	0.6808	0.7302	0.7096	0.7108

TABLE I(b)

Black cotton soil of Poona (maximum-minimum weight in gm.)

Depth of soil (mm.)	5-4-1939	6-4-1939	7-4-1939	8-4-1939	Mean
1	0.2748	0.2576	0.2828	0.2684	0.2079
2	0.3262	0.3140	0.3462	0.3624	0.3372
3	0.4608	0.4462	0.4470	0.4932	0.4618
4	0.5542	0.5984	0.5296	0.5998	0.5705
5	0.7630	0.7958	0.7428	0.7065	0.7520
10	0.9436	0.9920	0.9912	0.9296	0.9641
20	0.9008	0.9546	0.9008	0.8994	0.9139
40	0.9088	0.9420	0.8600	0.8784	0.8973

TABLE I (c)

Black cotton soil of Poona (maximum-minimum weight in gm.)

Depth of soil (mm.)	24-4-1939	25-4-1939	26-4-1939	Mean
1	0.2488	0.2452	0.1592	0.2177
3	0.5964	0.5028	0.3600	0.4864
5	0.8838	0.7320	0.5244	0.7135
10	0.9242	0.7572	0.5264	0.7363
15	0.9468	0.7304	0.4816	0.7229
20	0.9024	0.7288	0.4672	0.6995
25	0.8876	0.7268	0.4680	0.6941
30	0.8874	0.7244	0.4660	0.6926
35	0.8748	0.7195	0.4604	0.6849
40	0.8420	0.7150	0.4610	0.6727

TABLE I (d)

Black cotton soil of Poona (maximum-minimum weight in gm.)

Depth of soil (mm.)	1-5-1939	2-5-1939	3-5-1939	Mean
1	0.2104	0.2407	0.1836	0.2116
3	0.3263	0.3486	0.3046	0.3265
5	0.6810	0.6948	0.6624	0.6794
10	0.8506	0.8876	0.7060	0.8147
15	0.8300	0.8596	0.6908	0.7901
20	0.8108	0.8132	0.6824	0.7688
25	0.8054	0.8084	0.6788	0.7642
30	0.7986	0.8002	0.6742	0.7577
35	0.7914	0.7908	0.6740	0.7521
40	0.7788	0.7826	0.6724	0.7446

(b) *Exchange of moisture by the red soil of Bangalore as shown by the maximum and minimum weights*

As in the case of Poona soil, the vessels filled with the red soil of Bangalore were exposed during the period 24th April 1939 to 3rd May 1939 and the maximum and minimum weights were determined by weighing at 6 A.M. and 2 P.M. respectively. Tables II(a) and (b) give the difference between the maximum and minimum weights in gm. for various depths.

TABLE II (a)

Red soil of Bangalore (maximum-minimum weight in gm.)

Depth of soil (mm.)	24-4-1939	25-4-1939	26-4-1939	Mean
1	0.0304	0.0238	0.0276	0.0273
3	0.0952	0.0556	0.0396	0.0635
5	0.1472	0.0968	0.0734	0.1058
10	0.2532	0.1826	0.1334	0.1897
15	0.3568	0.2320	0.1656	0.2515
20	0.3996	0.2736	0.1884	0.2872
25	0.3668	0.2584	0.1698	0.2650
30	0.3516	0.2472	0.1594	0.2527
35	0.3262	0.2398	0.1598	0.2419
40	0.3128	0.2308	0.1522	0.2319

TABLE II (b)

Red soil of Bangalore (maximum-minimum weight in gm.)

Depth of soil (mm.)	1-5-1939	2-5-1939	3-5-1939	Mean
1	0.0338	0.0280	0.0248	0.0289
3	0.0506	0.0564	0.0546	0.0539
5	0.0902	0.0946	0.1002	0.0950
10	0.1784	0.1806	0.1748	0.1779
15	0.2686	0.2752	0.2213	0.2550
20	0.3198	0.3244	0.2936	0.3126
25	0.3104	0.3128	0.2600	0.2944
30	0.3068	0.2898	0.2484	0.2817
35	0.2924	0.2880	0.2422	0.2742
40	0.2868	0.2864	0.2400	0.2711

(c) *Exchange of moisture by the alluvial soil of Lyallpur as shown by the maximum and minimum weights*

The vessels were filled with alluvial soil of Lyallpur and exposed during the period 5th April 1939 to 13th May 1939. The maximum and the minimum weights were determined by weighing these vessels at 6 A.M. and 2 P.M. respectively. Tables III (a) and (b) give the difference between the maximum and minimum weights in gm. for various depths of soil.

TABLE III (a)

Alluvial soil of Lyallpur (maximum-minimum weight in gm.)

Depth of soil (mm.)	5-4-1939	6-4-1939	7-4-1939	8-4-1939	Mean
1	0.0464	0.0260	0.0342	0.0346	0.0353
2	0.0520	0.0544	0.0668	0.0508	0.0560
3	0.0600	0.0816	0.0960	0.0764	0.0785
4	0.0834	0.1436	0.1618	0.1338	0.1307
5	0.1176	0.1840	0.1992	0.1536	0.1636
10	0.1347	0.2006	0.2312	0.1884	0.1887
20	0.1956	0.3208	0.3496	0.2774	0.2859
40	0.1856	0.3056	0.3304	0.2584	0.2700

TABLE III (b)
Alluvial soil of Lyallpur (maximum-minimum weight in gm.)

Depth of soil (mm.)	8-5-1939	9-5-1939	10-5-1939	11-5-1939	12-5-1939	13-5-1939	Mean
1	0.0260	0.0166	0.0280	0.0204	0.0276	0.0292	0.0246
3	0.0456	0.0964	0.0448	0.0548	0.0985	0.0684	0.0681
5	0.0828	0.1804	0.1322	0.0848	0.1626	0.1946	0.1396
10	0.1660	0.2028	0.1645	0.1147	0.2096	0.2648	0.1871
15	0.2072	0.2867	0.2004	0.1642	0.2996	0.3264	0.2474
20	0.2438	0.3404	0.2336	0.1818	0.3964	0.4022	0.2937
25	0.2546	0.3498	0.2390	0.1940	0.4092	0.4002	0.3075
30	0.2314	0.3444	0.2310	0.1832	0.3408	0.3796	0.2851
35	0.2014	0.3284	0.2272	0.1706	0.3246	0.3468	0.2665
40	0.2000	0.3022	0.2096	0.1602	0.3018	0.3242	0.2497

(d) *Exchange of moisture by the sandy soil of Trivandrum as shown by the maximum and minimum weights*

The vessels were lastly filled with sand and exposed to the open during the period 30th May 1939 to 2nd June 1939. The maximum and the minimum weights were determined by weighing these vessels at 6 A.M. and 2 P.M. respectively.

Table IV gives the difference between the maximum and the minimum weights in gm. for various depths of soil on different days.

TABLE IV
Sandy soil of Trivandrum (maximum-minimum weight in gm.)

Depth of soil (mm.)	30-5-1939	31-5-1939	1-6-1939	2-6-1939	Mean
1	0.0000	0.0000	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000	0.0000	0.0000
4	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.0003	0.0004	0.0000	0.0000	0.0002
10	0.0030	0.0042	0.0002	0.0002	0.0019
15	0.0289	0.0316	0.0162	0.0142	0.0227
20	0.0296	0.0380	0.0164	0.0148	0.0247
25	0.0388	0.0484	0.0246	0.0226	0.0341
30	0.0412	0.0508	0.0316	0.0302	0.0383
35	0.0464	0.0540	0.0398	0.0324	0.0431
40	0.0470	0.0548	0.0400	0.0328	0.0437

DISCUSSION

An examination of the above tables will show that on clear days the 'surface layer' of the soil is constantly exchanging moisture with the air layers near the ground. The amplitude of this moisture exchange is found to be greatest in the black cotton soil of Poona and least in the sandy soil of Trivandrum. Also the amplitudes of the soils studied are in about the same relation as found by Ramdas and Katti [1934, 2].

It is also seen from the tables that in all the soils examined the amount of moisture exchanged goes on increasing till a certain depth is reached beyond which the amplitude of variation does not show much change with further increase in the depth of soil.* The above depth indicates the actual thickness of the surface layer which is taking part in the diurnal exchange of moisture between the soil and the air layers near the ground. The exact depth of soil involved is different in the different types of soils. Table V gives the depth of soil exchanging moisture with the atmosphere in the case of the four types of soils studied.

TABLE V

Type of soil	Depth of soil involved in the diurnal exchange of moisture (mm.)	Mean maximum amplitude of variation in weight gm. per sq. cm.
1. Black cotton soil of Poona	10	0.0460
2. Red soil of Bangalore	20	0.0169
3. Alluvial soil of Layallpur	25	0.0167
4. Sandy soil of Trivandrum	More than 40	0.0025

Thus it is seen that although the amount of moisture exchanged is maximum, the depth to which this moisture exchange extends is minimum in the Poona soil. On the other hand, in the sandy soil of Trivandrum, the moisture exchange penetrates much deeper although the amount of moisture exchanged is very small. As is well known, the black cotton soil contains a very high clay fraction and is therefore the least porous, whereas the sandy soil of Trivandrum is free of clay and is therefore the most porous of the soils examined. It appears, therefore, that the exact depth of soil involved in the diurnal exchange of moisture increases with the porosity of the soil.

*From the tables it will be noticed that there is a slight tendency for the difference between the maximum and the minimum weights of the soils to decrease with depth below the depth of maximum difference. This secondary effect was noticed systematically in all the experiments and is presumably due to heat conducted to the interior layers of the soil samples through the sides of the metallic vessels. This secondary effect will be examined in detail during the next clear season.

SUMMARY AND CONCLUSIONS

On clear days there is an exchange of moisture between 'surface layer' of soil containing hygroscopic moisture only and the air layers near the ground. The amplitude of the diurnal exchange of moisture is maximum in the black cotton soil of Poona and minimum in the sandy soil of Trivandrum.

The depth to which this moisture exchange extends is different in different soils being smallest in the black cotton soil of Poona and greatest in the sandy soil of Trivandrum, the red soil of Bangalore and the alluvial soil of Lyallpur having intermediate values.

Experiments with the different components of the soil obtained by mechanical analysis will be undertaken during the next clear season.

My best thanks are due to Dr. L. A. Ramdas, Agricultural Meteorologist, for suggesting the problem and for guidance during the course of the work.

REFERENCES

- Katti, M. S. (1935). *Current Sci.* **4**, 419
Ramdas, L. A. (1934). *Current Sci.* **2**, 445
Ramdas, L. A. and Katti, M. S. (1934, 1). *Ind. J. Agric. Sci.* **4**, 923
————— (1934, 2). *Current Sci.* **3**, 24
————— (1935). *Current Sci.* **3**, 612
————— (1936). *Ind. J. Agric. Sci.* **6**, 1163
Ramdas L. A. and Mallik, A. K. (1938). *Current Sci.* **6**, 452
————— (1939). *Current Sci.* **4**, 164

CONVERSION OF CANE MOLASSES INTO MANURE BY THE BIOLOGICAL METHOD AND THE RESULTS OF THE CROPPING TESTS WITH THE MANURES PREPARED (1938-39)

BY

H. D. SEN, M.Sc., PH.D.(LOND.), D.I.C.

Biochemist, Imperial Institute of Sugar Technology, India, Cawnpore

(Received for publication on 10 August 1939)

THE application of molasses to soil for use as manure has been a subject of research for a fairly long time and the uncertainty in the results so far obtained by different workers may be ascribed to the fact that molasses on fermentation in the soil lead to the production of acids, which retard the plant growth. In cases of application of molasses in soil in comparatively small doses, where the alkalinity of the soil is sufficient to cope with the acid produced as a result of fermentation, deleterious effect is not so very evident, but when molasses is applied in heavy doses the depressant action is very significant.

Gainy [1923], working on the influence of hydrogen-ion concentration on the growth and fixation of nitrogen by cultures of *azotobacter*, finds pH 5.9 to 6.0 as the limiting pH below which no fixation of nitrogen can take place. Soil having acidity below 6.0 showed complete absence of *azotobacter*. If, however, calcium carbonate is added to reduce the acidity, such soil was found to favour the growth of *azotobacter*. Addition of more acids leads to its complete disappearance.

Hence it is very likely that when molasses is applied directly into the soil, particularly in heavy doses, there will be nitrogen loss instead of nitrogen fixation due to greater acid accumulation.

Paramanik, Rao and Lal [1937], working on cane yield, observed the beneficial effect of using molasses along with ammonium sulphate, particularly in alkaline soils. This leads one to conclude that whereas there is nitrogen loss when heavy dose of carbohydrate material is applied, this is partially avoided when the carbohydrate is applied in conjunction with a certain proportion of nitrogenous material, i.e. there is a carbon-nitrogen (C : N) ratio.

The mechanism of reaction taking place in the soil on direct application of molasses seems, therefore, to be very complicated, which is rendered more unsurmountable by the varied soil flora present—the reactions taking place leading to uncertain results—sometimes nitrogen fixation and in other cases nitrogen loss.

One would naturally ponder whether molasses has a potential value as manure or its biological products—yeast, salts of organic acids and nitrogenous decomposition products.

Viswanath and Suranarayana [1932], working with *Halianthus annus* of the giant yellow variety got striking results by directly injecting aqueous extracts of dried yeast, farmyard manure and sewage effluent.

Narayana [1932] pointed out that extracts of fermented farmyard manure are more effective in stimulating plant growth than fresh farmyard manure.

Desai and Fazal-ud-Din [1938] have carried out experiments in which the presence of yeast has led to the thriving of symbiotic soil flora, such as *Colstrydium acetobutylicum*, in soil. These do not thrive on carbohydrate materials as such, but become active in presence of yeast.

Bhaskaran and Subramanyan [1937] showed that, whereas organic acids have definite deleterious effect, their calcium salts led to greater nitrogen fixation.

Tinkler [1937] stated that apex of rootlets produce substances capable of regulating the growth and that these substances could be transferred to other seedlings. A wide search for a ready source of these compounds revealed that they are contained in small quantities of urine.

Kogl [1937] established the constitution of the active substance to be of the class of indolyl acetic, propionic, butyric acids, etc. and their esters. Skatole which is β methyl indole has been proved to be active and so are α and β naphthelene acetic acids.

The fermented molasses solution contains yeast, calcium salts of acetic, propionic, butyric acids, etc. and also nitrogen decomposition products, such as indole, acetic, butyric, propionic acids and their esters and also skatole and as such are likely to be more beneficial to plant growth than molasses itself.

The main difficulty in the use of molasses directly in the soil lies in the fact that it is to be applied in a diluted form and a large bulk of water is necessary for the purpose. The application of such diluted solution in the field invariably spreads an unbearable smell all along the water channel leading such polluted water, and particularly in cases where there is an accumulation of dilute molasses solution in pits.

The complex bacterial population of the soil and the uncertain results that might happen if molasses is directly applied and the problem of conversion of large quantities of molasses into manure in a comparatively short time led the author to devise a simple method, the details of which will be discussed in the present paper.

The working of the process depends on the increased production of yeast cell body by carrying on fermentation at the neutral point under conditions of heavy aeration the acidity being intermittently neutralized with milk of lime. The course of fermentation under the above circumstances is diverted not to the production of alcohol and carbon dioxide but to the theoretical preliminary formation of aldol and subsequent formation of products like glycerol which is further taken up by the yeast cells to increase their body. Thus increased yield of yeast is obtained with simultaneous production of calcium salts of organic acids.

The prevention of loss of nitrogen by administering molasses along with ammonium sulphate or the existence of carbon-nitrogen ratio may be explained by the theory that as a result of fermentation of sugars present in molasses yeast is initially formed, which is produced in increased yield due to the application along with it of small quantities of ammonium sulphate or phosphate, acting as nutrient.

That aeration with neutralization of the acidity so as to maintain a pH of 7.0 during fermentation gives increased yield of yeast has been confirmed by a study of the yeast yield where instead of milk of lime sodium carbonate solution was added to neutralize the acidity. The addition of 1.5 per cent of ammonium sulphate gave definite increased yeast yields (about three times the control) while addition of ammonium phosphate gave the highest yield (about six times). Thus it seems that there is not only carbon-nitrogen ratio but carbon-nitrogen-phosphorus ratio.

Although the duration of aeration in the preliminary experiments was eight hours every day for three days, i.e. 24 hours, an aeration for a period of 72 hours, i.e. continued aeration for three days was found to be most beneficial.

Process of manufacturing manure from molasses

The process of manufacturing manure from molasses consists in constructing three masonry tanks 10-ft. \times 10-ft. \times 8-ft. (4,984 gallons) having pipe lines from an air blower, the main pipe line being 2-in. in diameter, connected to three parallel 1-in. pipe lines in the middle of each tank. The central 1-in. pipe line in each tank has three $\frac{1}{2}$ -in. pipe lines running at right angles to it and bending downwards to 1-ft. from the base so as not to disturb the sludge collected at the bottom. Each tank is provided with a centrifugal pump for the transference of the fermented liquor to the next tank.

Seventy-five maunds of molasses (88 brix) is diluted with 3,000 gallons of water (13 brix) in the first tank to which 75 maunds of filter press mud is thoroughly incorporated. Sixty gallons of active wash made three days earlier by adding to a solution of 1 md. of molasses in 60 gallons of water in a wooden cask of that capacity to which 12 gallons ($\frac{1}{5}$ th) of active wash (prepared from pure yeast, *S. Ellipsoidus*, in the Laboratory) is added. This is propagated in six casks containing 360 gallons of active wash. One-fifth is left in the fermenting casks. Two hundred and forty gallons (5 maunds molasses) is added to the wash prepared above. Half a maund of ammonium phosphate (Nicipos of Imperial Chemical Industries containing 17 per cent nitrogen and 17 per cent phosphorus is added to the wash and aeration started with intermittent addition of lime (12 Be) every four hours. At the end of 24 hours' aeration the fermented liquor is transferred to the second tank leaving the sludge, a second dose of $\frac{1}{2}$ md. ammonium phosphate added and aeration started with intermittent addition of lime every four hours. In the meantime the first tank is charged with fresh 75 mds. of molasses and 75 mds. of filter press cake and 3,000 gallons of water. Two hundred and forty gallons of active wash is further added with $\frac{1}{2}$ md. ammonium phosphate and aeration started in the first tank for 24 hours. On the third day the wash from the second tank is transferred to the third tank and the wash from the 1st tank transferred to the second.

A fresh charge of 75 mds. of molasses and 75 mds. of filter press cake in 3,000 gallons of water and 240 gallons of active wash (5 mds. molasses) is made on the third day in the first tank and $\frac{1}{2}$ md. ammonium phosphate added. The first charge of liquor which has now reached the third tank and completed three days' continued aeration is run into the fields having also considerable manurial value. The second charge of fermented wash is transferred to the third tank and $\frac{1}{2}$ md. ammonium phosphate added and aerated for 24 hours.

On the fourth and fifth days no fresh charge of molasses is made but there is transference of the third charge into the second and third tanks.

On the sixth day the sludge collected in all the three tanks is transferred to shallow cemented masonry beds for sun-drying and manure eventually packed in bags and sent to the cane fields. The fermented liquor, which is quite harmless after treatment, is sent through water channels to the cane fields. At the end of a week thus 240 mds. of molasses and an equal quantity of filter press mud will receive treatment yielding about 40 per cent manure on the total weight, i.e. 172 mds. besides 9,000 gallons of fermented liquor having high manurial value.

Expenses—		Rs.	As.
Cost of exhaust molasses 240 mds. @ 4 annas per md.	.	60	0
Filter press cake @ 6 pies per md.	.	7	8
Nicifos @ 6/- per md, 4½ mds. containing 17 per cent N and 17 per cent P	.	27	0
Lime 40 mds. @ 8 annas per md.	.	20	0
Electricity @ 1 anna per unit 24 KWH.	.	1	8
Labour, 2 coolies @ 5 annas per day for six days	.	3	12
Total Rs.		119	12

∴ Cost of 170 mds. of manure (N. 1.0 per cent) 119-12 = Rs. 120.

∴ Cost per md. of manure—/11/3 = 12 annas only.

EXPERIMENTAL

Sen and Dutta [1937] and Sen [1938] have studied the nitrogen distribution in the sludge and the fermented liquor where 50 mds. of molasses was diluted with 6,000 gallons of water under conditions where acids were allowed to accumulate as compared with those where the acids were neutralized with milk of lime (12 Be) using a mixed bacilli obtained by self-fermentation of molasses consisting of *B. coli*, yeast, acetic, and lactic bacteria. In case of acid fermentation the supernatant fermented liquor was taken to an adjoining tank and neutralized with lime. The sludge separated in both tanks were collected, sun-dried, weighed and analysed. The data obtained are shown in tables I(a)-(c).

TABLE I (a)

Description	Weight taken	Dry matter per cent	Total N per cent	Total N in dry substance in lb.
Original molasses	50 mds.	73.8	0.514	15.64
1. Manure by acid fermentation	10—10	94.7	1.684	13.17
2. Manure from chemical treatment tank	21—00	100.0	0.0043	0.07
3. Exit Water	6,000 gall.	..	2.6 parts per 100,000 parts	1.56 — — — — 14.80

TABLE I (b)

Description	Weight taken	Dry matter per cent	Total N per cent	Total N in dry substance in lb.
Original molasses	50 mds.	73.8	0.514	15.64
1. Manure obtained by acid fermentation	11 mds.	100.0	1.54	13.94
2. Manure obtained from chemical treatment tank	18 mds., 8 srs.	100.0	0.14	2.06
3. Exit water	6,000 galls.	..	2.5 parts in 100,000 parts	1.50 <hr/> 17.50

TABLE I (c)

Fermentation with intermittent neutralization with milk of lime

Original molasses	50 mds.	73.8	0.514	15.64
1. Manure by intermittent addition of lime with heavy aeration	24 mds., 20 srs.	100.0	1.23	24.0
2. Exit water	6,000 galls.	..	8.24 parts in 100,000 parts	4.9 <hr/> 28.94

The above figures indicate that, in cases where fermentation is carried out without aeration, organic acids are allowed to accumulate and the wash is saturated with carbon dioxide gas, only 84.0-89.1 per cent of the total nitrogen present in the original molasses is fixed in the sludge, 10.4 per cent remain in the fermented liquor and the rest is lost; while in cases of intermittent neutralization with heavy aeration there is distinct evidence of nitrogen fixation, the total increase in the sludge and the fermented liquor being 185 per cent, i.e. nearly double the original nitrogen content.

Effect of using pure yeast instead of mixed bacteria

In the later experiments it was found that the nitrogen content of the sludge increased definitely by using a pure culture of yeast instead of a mixed bacteria. The percentage of nitrogen in the sludge increased where there was preponderance of yeast cells. Whereas by using mixed bacteria with intermittent addition of lime a sludge containing 0.92 per cent nitrogen was obtained, pure yeast gave a sludge with a nitrogen content of 1.84 while a sludge

with a nitrogen content of 2.1 per cent was obtained when equal mixtures of molasses and filter press cake were fermented with lime addition. The waxes, gums and organic matters present in the filter press cake gave comparatively more nutrient for the growth of the yeast body.

The nitrogen balance in molasses and manure obtained from a mixture of molasses and press cake using pure yeast are given in table II.

TABLE II

Fermentation of a mixture of molasses and filter press cake with aeration and intermittent addition of lime using pure yeast

Description	Weight taken (mds.)	Dry matter per cent	Dry matter (md.)	Nitrogen per cent on dry matter	Total N in dry substance in mds.
Molasses	50	82.0	41	0.27 (on wet molasses)	0.1107
Press mud	50	30.0	15	0.9	0.0014
					0.1121
1. Manure with intermittent lime addition and aeration	53	33.0	17.6	1.46	0.25
2. Exit water	6,000	9.9 parts in 100,000 parts	0.072
					0.322

There is thus evidence of still greater nitrogen fixation when molasses is used in conjunction with filter press cake using pure yeast.

Yield of yeast when fermentation is carried out at the neutral point with aeration

To ascertain whether by carrying out the fermentation at the neutral point, there is increase in the yeast yield a series of experiments were undertaken by Sen and Dutta [1938]. In these experiments milk of lime was replaced by a solution of sodium carbonate so that the results may not be vitiated by the presence of calcium salts. The yeast obtained was separated by centrifuging and wet yields (moisture 50 per cent) were determined after washing the yeast sediment twice with distilled water to remove the sodium salts. The data obtained are given in table III.

TABLE III

Percentage yield of manure obtained at different specific gravities calculated on molasses and total sugars assuming that molasses contain 60 per cent sugars

Treatments	Specific gravity 1.0528				Specific gravity 1.0340				Specific gravity 1.0178			
	Weight* of sediment in 100 c.c. (gm.)	Per cent yield on sugars 12 gm. present in 100 c.c.	Per cent yield on molasses 20 gm. present in 100 c.c.	Weight* of sediment in 100 c.c. (gm.)	Per cent yield on molasses 6.6 gm. present in 100 c.c.	Per cent yield on molasses 11 gm. present in 100 c.c.	Weight of sediment in 100 c.c. (gm.)	Per cent yield on sugars 5 gm. present in 100 c.c.	Weight of sediment in 100 c.c. (gm.)	Per cent yield on sugars 5 gm. present in 100 c.c.	Per cent yield on molasses 8.4 gm. present in 100 c.c.	Per cent yield on molasses 10 gm. present in 100 c.c.
1. Control— Fermentation without aeration or neutralization	1.126	9.4	5.6	1.765	26.7	16.0	1.431	28.6	1.431	28.6	17.0	
2. Fermentation with aeration and neutralization with sodium carbonate	1.824	15.2	9.1	2.339	35.4	21.2	1.741	34.8	1.741	34.8	20.7	
3. Fermentation with aeration and neutralization with sodium sulphite	1.478	12.3	7.3	2.332	34.5	21.1	2.214	44.2	2.214	44.2	26.3	
4. Fermentation with ammonium sulphate and aeration and neutralization with soda	3.372	28.1	16.8	3.321	50.0	30.1	2.705	54.1	2.705	54.1	32.2	
5. Fermentation with ammonium phosphate 1 per cent neutralization with soda	6.723	56.1	33.6	8.346	126.4	75.8	8.11	162.0	8.11	162.0	96.5	

*moisture 50 per cent

The results indicate that :

1. The yield of yeast is least when the fermentation is carried out without aeration or intermittent neutralization of sodium carbonate.

2. The yield of yeast increases about $1\frac{1}{2}$ times under aeration and intermittent neutralization with sodium carbonate during 72 hours.

3. The increase in the yeast production is not so much when neutralization is carried out with sodium sulphite although it is decidedly greater by at least one third the yield obtained without aeration or neutralization.

4. The use of ammonium sulphate 1.5 per cent on molasses augments the yield to about three times that obtained without aeration or neutralization.

5. The use of ammonium phosphate as nutrient in the proportion of 1.5 per cent molasses increases the yield to about six times that obtained without aeration or neutralization.

6. The yield of yeast increases with increasing dilution of molasses solution. Molasses solution fermented as 1.017 gave yeast yield two to three times as great as that obtained at 1.052. Molasses solution fermented at 1.034 gave yield twice as great.

The preliminary experiments show that yeast yield is definitely increased when fermentation is carried out at the neutral point with aeration and that ammonium phosphate and ammonium sulphate (1.5 per cent) augment it considerably (six to three times), the former having more marked result.

On the basis of the above experiments manures have been prepared from molasses under the following conditions :—

1. Molasses fermented with intermittent addition of lime at the interval of four hours with continuous aeration during 72 hours.

2. Molasses and filter press cake mixed in equal proportions fermented with intermittent addition of lime at the interval of every four hours with continuous aeration during 72 hours.

A portion of the fermented liquor after separation of the sludge was concentrated in each case. Samples of the sludge and fermented wash concentrates have been set apart for complete analysis of organic and inorganic constituents. The analyses are in progress.

A qualitative micro-test of the samples will reveal whether besides yeast there are present calcium salts of acetic, propionic, butyric acids and also indol and skatole, acetic, propionic, butyric acids or their esters which are active plant hormones.

An investigation is also in progress to determine how the course of fermentation is diverted under conditions of heavy aeration and constant neutralization of the acidity with alkali salts. It is presumed that under the above conditions the course of fermentation is diverted leading to less production of alcohol and more of yeast cell body.

CROPPING EXPERIMENTS WITH THE MANURES PREPARED FROM MOLASSES 1938-39 EXPERIMENTS

Mr. P. B. Richards, I. A. S., Director of Agriculture, United Provinces, took a great interest in the newly prepared manure and allotted a piece of land, about three acres at the Kalyanpur farm, United Provinces. The plot selected had not received any manurial treatment.

There were nine treatments with six replications 40 ft. \times 31.5 ft. and each plot split into three sub-plots having three varieties of cane—Co 313, Co 312 and Co 331—early, medium and late varieties. The manures were applied in randomised blocks distributed according to the schemes shown below.

The distance between two sub-plots was 7 ft. longitudinally and 5 ft. on the broad side. Two rows of cane between varietal treatment in each sub-plot were left out during harvesting, while two rows in between two sub-plots received no manurial treatment. The area of each sub-plot was 1/30th of an acre which was sub-divided into three smaller plots having different cane variety, and measuring 1/90th of an acre.

Particulars

Date of sowing	2nd March 1938
Previous irrigation to sowing	20th February 1938
After sowing, irrigation on	6th March 1938
	3rd April 1938
	29th April 1938
	22nd May 1938

Area of plots.—Length 46 ft. \times breadth 31.5 ft.

Rows in each.—9. Three rows of each variety in treatment.

Treatment A.—Molasses fermented with lime to give 60 lb. nitrogen per acre.

Treatment B.—Molasses fermented with lime to give 120 lb. nitrogen per acre.

Treatment C.—Molasses *plus* filter press cake fermented with lime, 60 lb. nitrogen per acre.

Treatment D.—Molasses *plus* filter press cake fermented with lime, 120 lb. nitrogen per acre.

Treatment E.—Molasses (direct application) to supply 60 lb. nitrogen per acre.

Treatment F.—Molasses (direct application) to supply 120 lb. nitrogen per acre.

Treatment G.—Castor cake to supply 60 lb. nitrogen per acre.

Treatment H.—Castor cake to supply 120 lb. nitrogen per acre.

Treatment I.—Control.

a—Co 313, early-ripening variety.

b—Co 312, medium-ripening variety.

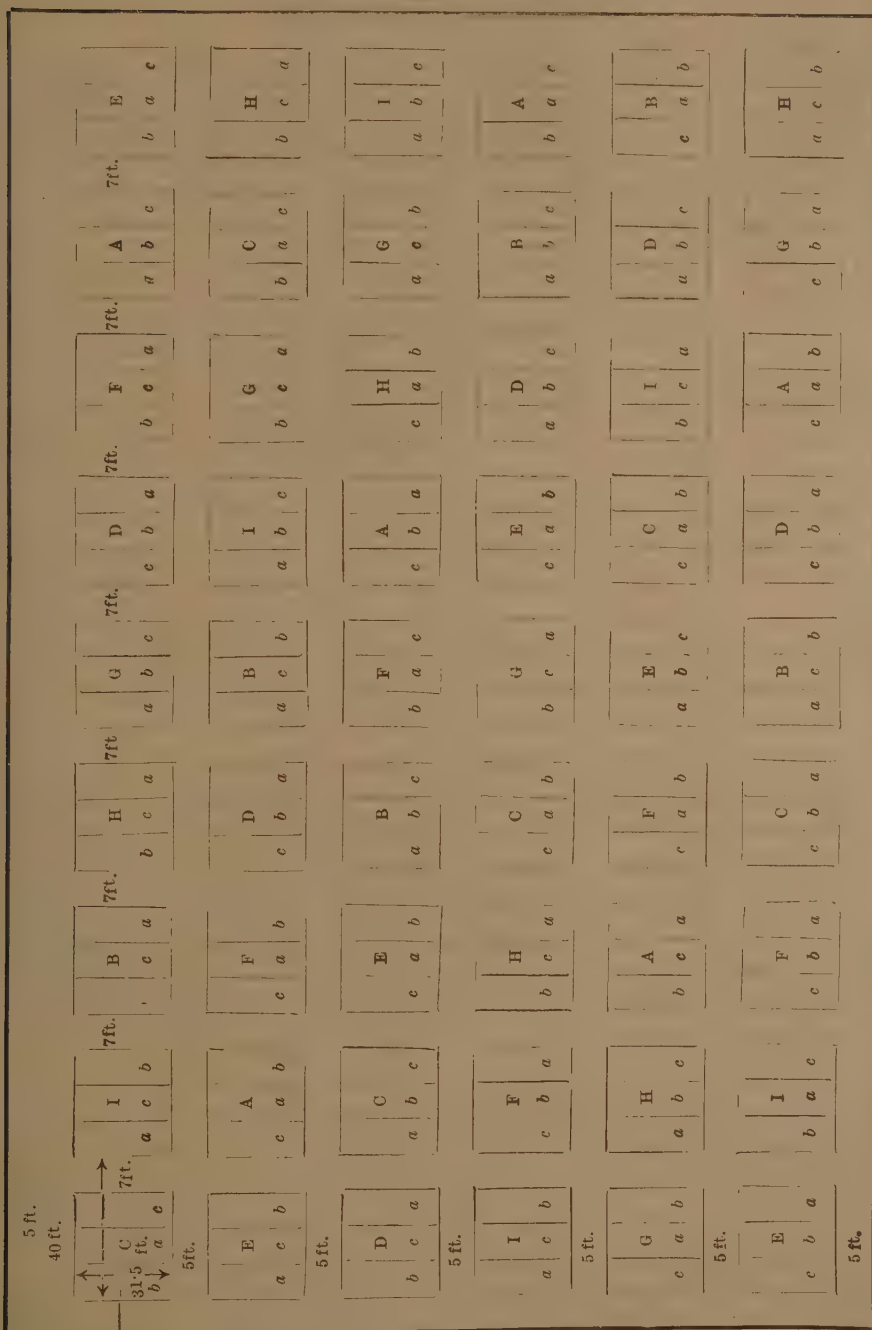
c—Co 331, late-ripening variety.

The plots being 1/30th acre, molasses (0.25 per cent nitrogen) was added at 10 mds. and 20 mds. respectively to supply 60 and 120 lb. nitrogen. The prepared manures (calculated taking nitrogen content at 1.0 per cent) were added in the proportion of 2.5 and 5 mds. respectively to supply 60 and 120 lb. nitrogen per acre. Castor cake (4 per cent nitrogen) was supplied at 0.75 and 1.25 mds. respectively.

The pH of the Kalyanpur soil, as determined by the electrolytic method was found to be 7.3 while Hillige's comparator gave 7.3. The soil may, therefore, be taken as almost neutral soil with slight leaning towards alkalinity.

East

North



South

West

PRELIMINARY OBSERVATIONS

Rate of growth

Systematic measurements of the seedlings were made in the months of June and July 1938 to study the comparative response to different manures. Six seedlings from each sub-plot were carefully measured and the mean height for six replications was recorded. In each case the measurement (in feet) was made from the root to the top of the stalk.

TABLE IV
Measurement of seedlings in feet

Treatments	Co 313			Co 312		Co 331	
	May	June	July	June	July	June	July
A .	10.63	16.0	52.4	18.6	57.3	14.07	53.2
B .	10.62
C .	13.5	15.5	51.4	18.7	56.0	14.3	52.9
D .	12.87	18.7	51.4	17.9	57.0	14.6	53.3
E .	11.91	15.9	51.0	16.9	56.6	14.7	52.5
F .	11.62	12.6	48.6	13.4	51.0	12.4	51.6
G .	11.63	15.4	53.5	18.1	57.8	15.3	52.3
H .	15.91	16.4	57.3	21.9	64.5	16.5	52.3
I .	12.7	13.7	50.7	16.4	55.0	13.7	50.8

On studying the rate of growth of cane seedlings it may be noticed that direct application of molasses has a definite retarding effect, especially in treatment F when molasses is applied at the rate of 120 lb. nitrogen per acre although the retarding effect is not so evident in treatment E when molasses is used in small doses that is 60 lb. nitrogen per acre. The rate of growth is much less than even the control plot. Manures A, B, C and D prepared by the biological method from molasses and also from mixtures of molasses and filter press cake show no retarding effect. On the contrary it had definite beneficial effect even when applied in as high dose as 120 lb. nitrogen per acre.

Rate of germination

The observations made by the Superintendent, Kalyanpur Farm on the rate of germination are :—

Treatments . . .	A	B	C	D	E	F	G	H	I
Percentage of germination .	Good	Good	Fair	Good	Fair	Bad	Good	Good	Fair

Susceptibility to disease

The observations made by the Superintendent, Kalyanpur Farm are given in Table V.

TABLE V

Treatments	Co 313	Co 312	Co 331
A molasses manure 60 lb. N per acre	Fair growth, better than control, tillering good	Fair growth, internodes shorter	Stunted canes, fair tillering, more leafy growth
B molasses manure 120 lb. N per acre	Growth very good, tillering same as castor cake	Very long canes, less leafy growth, average tillering	White ant attack marked throughout, lodging at places, canes long
C molasses + filter press cake, 60 lb. N per acre	Growth poorer, mosaic infection at places, tillering average	Tillering better	Mosaic attack, tillering average
D molasses + filter press cake, 120 lb. N per acre	Canes thick, development fair, leaf not healthy	Lodging, very long canes, tillering very good	Leafy growth, shorter canes, lodging, tillering fair
E molasses, direct application, 60 lb. N per acre	Growth poor, leaves pale and sickly, tillering average similar to control	Growth poor, root system weak, tillering poor	Termite attack vigorous
F molasses, direct application, 120 lb. N per acre	White ant attack, development bad	White ant attack, lodging throughout	Leaves paler at tops, shows lack of nutrition, lodging
G castor cake, 60 lb. N per acre	Good leafy growth, tillering fair, no lodging	Fair and less leafy growth	Canes fairly long
H castor cake, 120 lb. N per acre	Well developed good growth, tillering average	Best growth, lodging at places	Very good growth, lodging at places
I control	Poor growth and tillering	Growth and tillering poor	Tillering poor, top borer attack

The data obtained confirm the observation that treatments E and F (direct molasses application) gave poorer growth, sickly leaves, white ant and termite attack. The growth with prepared manures (A, B, C, D) is generally good although with Co 331 there has been evidence of white ant and mosaic attack. Each variety Co 313, Co 312, and Co 331—early, medium and late varieties—was tested for maturity by the study of the top-middle-bottom brix and purity ratio of the juice. When the ripening tests were satisfied each cane variety was analysed for total sucrose in cane and the average of six replications was taken into account for calculation. The harvesting was undertaken soon after the analysis and the average of six replications determined. Co 313 was harvested on 26th January 1939, Co 312 on 21st February 1939 and Co 331 on 1st March 1939.

Calculations were made for each cane variety on the basis of the total sucrose in cane and cane yield obtained as average of six replications of cane yield per acre, sucrose per acre, excess of cane yield per acre over control, excess of cane yield per acre over F (molasses, direct application in heavy doses @ 120 lb. nitrogen per acre), excess sugar yield per acre over control, and excess of sugar yield per acre over F.

TABLE VI

Co 313—EARLY RIPENING VARIETY.

*Percentage of total sucrose in cane obtained under different manurial treatments ;
Co 313 : tests carried out after verification of the maturity by the top-
bottom-brix ratio and also top-bottom-purity ratio by Java method*

Treatments	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Average	Increase or decrease over control
A	14.48	14.21	14.14	14.15	13.4	14.57	14.13	+1.14
B	14.30	14.50	13.82	15.07	12.73	12.61	13.83	+0.83
C	13.91	14.74	14.75	12.84	12.72	13.86	13.80	+0.81
D	13.21	13.47	13.98	11.26	13.11	12.90	12.98	—0.01
E	13.30	13.34	14.29	11.63	13.28	12.62	13.07	+0.08
F	13.93	13.44	13.30	11.78	11.48	14.04	12.98	—0.01
G	14.35	13.52	14.16	9.80	13.75	14.39	13.32	+0.33
H	12.28	13.78	12.88	13.46	13.89	14.10	13.39	+0.40
I	13.22	11.88	13.51	14.98	11.70	12.70	12.99	...

The percentage of total sucrose in cane, taking the average of six replications, varies from 12.98 to 14.13, i.e. by 1.13 per cent only. It may, therefore, be said that on maturity, the percentage of total sucrose in cane tends to a maximum constant figure irrespective of manurial treatments. The four treatments with molasses manure A, B and C, however, show slight increase. With D it is the same as control. Molasses (direct application) in light and heavy doses E and F have given the same total sucrose as the control. The increase in case of castor cake treated plots G and H, though higher than the control is nearly half the increase obtained in the case of concentrated manures.

TABLE VII

Yield of cane per plot 1/90th acre. Co 313 (Harvested on 26th January 1939)

Treatment	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Average
	Mds.	Mds.	Mds.	Mds.	Mds.	Mds.	Mds.
A	8—31	7—29	8—33	8—11	6—31	8—2	8—2
B	7—33	8—5	8—10	8—4	8—0	7—1	7—35
C	7—16	6—11	7—2	7—36	8—17	6—2	7—20
D	7—2	7—8	6—37	8—17	7—33	7—27	7—20
E	6—35	8—11	9—24	9—16	9—17	8—29	8—28
F	4—18	4—25	3—8	6—15	5—18	7—27	5—15
G	8—10	6—16	6—28	8—34	7—34	8—3	7—27
H	8—13	7—32	7—17	9—19	8—18	8—26	8—14
I	6—14	6—12	7—20	6—18	7—34	7—0	6—15

The cane yield in 1/90th acre plots shows a definite depressant effect in plot F treated with heavy dose of molasses (1 md. 20 srs.). The concentrated manures A, B, C, D show uniformly an increase over the control plot (+1 md. 25 srs., +1 md., +25 srs., +25 srs.). The deleterious effect is not observed in plot E, treated with light dose of molasses (+1 md. to 33 srs.). The cane yield in case of castor cake is nearly the same as the concentrated manures.

TABLE VIII

Calculations on the cane yield and total sucrose per acre—Co 313

Treatments]	Canes yield in 1/90th acre	Canes yield per acre	Per cent sucrose	Sucrose per acre	Excess yield per acre over control	Excess cane yield per acre over F	Excess sugar yield per acre over control	Excess sugar yield per acre over F
	Mds.	Mds.		Mds.	Mds.	Mds.	Mds.	Mds.
A	8—2	724·5	14·13	102·47	+130·8	+241·5	+22·59	+39·88
B	7—35	707·75	13·83	98·02	+93·55	+214·75	+18·14	+35·43
C	7—20	675·0	13·80	93·45	+61·30	+192·0	+13·57	+30·86
D	7—20	675·0	12·98	87·61	+61·30	+192·0	+7·73	+25·02
E	8—28	783·0	13·07	103·33	+169·3	+300·0	+23·45	+40·76
F	5—15	483·0	12·98	62·59	—130·7	...	—17·29	...
G	7—27	690·75	13·32	92·00	+77·0	+207·75	+22·12	+29·41
H	8—14	751·5	13·39	100·57	+138·0	+268·5	+20·69	+37·98
I	6—35	613·72	12·99	79·88	...	+130·7	...	+17·98

Thus the concentrated manures A, B, C and D have given an increase in the cane yields per acre by nearly 100 mds. as compared with the control (+111·0, +94·0, +61·3 and +61·3 mds.) and 200 mds. as compared with molasses in heavy doses (+261·5, +227, +182 and +182 mds.). Molasses (direct application) in small doses does not show any deleterious effect (+170 mds. more than control, +300 mds. more than F). Molasses in heavy doses show definite depressant effect (—110 mds. less than the control). Castor cake behaves similarly to the concentrated manures (+87, +148 mds. respectively as compared with control and +207 and +268 mds. respectively as compared with F).

The increase in the total sucrose yield per acre with concentrated manures comes to about 20 mds. (+22·59, +18·14, +13·57 and +7·73) as compared with the control while molasses, direct application in heavy doses (F), give a deficit of 17·29 mds. The deleterious effect of molasses is not evident when applied in light doses (E) the increase in yield of sugar per acre being 23·45 mds. Castor cake gave 22·12 and 20·69 mds. increase respectively over the control. Comparing with F (molasses in heavy doses) there is increase in yield in cases of A, B, C, D treatments (+39·88, +35·43, +30·86 and +25·82 mds. respectively) and so with castor cake (+29·41, 37·98 mds.). Even the control shows an increase by 17·98 mds.

TABLE IX

Co 312—MEDIUM-RIPENING VARIETY

*Percentage of total sucrose in cane obtained under different manurial treatments
Tests carried out after verification of the maturity*

Treatments	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Average
A	9.0	12.70	12.99	11.9	13.94	12.79	12.22
B	11.72	7.00	13.41	12.19	10.69	11.52	11.09
C	12.10	10.12	12.84	12.80	13.79	11.99	12.27
D	12.81	10.75	13.40	13.06	13.66	9.61	12.21
E	12.49	11.65	12.84	12.98	12.92	12.26	12.52
F	12.44	12.55	12.52	12.35	11.89	12.15	12.31
G	10.19	13.55	13.49	11.91	7.46	13.14	11.62
H	11.92	11.77	7.10	10.88	9.24	12.73	10.56
I	9.31	13.07	13.08	13.69	12.79	12.90	12.47

Taking the cane variety Co 312 the total sucrose in cane is found to be more or less the same as control and is not affected by various manurial treatments. In Co 313 some differences, though very slight, was discernable. The castor cake treated plots, however, show a definite lowering due to more leafy growth.

TABLE X

Harvesting of Co 312 (1/90 acre)

Treatments	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Average
	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
A	11—26	11—3	12—15	11—37	12—32	10—1	11—26
B	10—37	12—0	12—1	11—18	12—17	12—30	11—37
C	10—18	10—8	10—21	16—11	11—11	10—27	11—23
D	9—3	10—12	10—30	10—25	13—15	11—15	10—37
E	9—6	10—9	12—17	11—24	10—24	11—16	10—36
F	8—25	8—0	9—37	7—20	7—12	4—38	7—28
G	10—24	10—11	13—2	10—6	10—10	13—10	11—10
H	10—10	6—8	10—28	12—8	10—33	13—12	10—23
I	10—1	10—9	10—23	10—10	10—28	10—22	10—15

Taking the average of six replications the cane yields with concentrated manures A, B, C and D are definitely greater than the control (+1 md. 11 srs., +1 md. 22 srs., +1 md. 3 srs. and +22 srs.) and greater than the plot F with heavy molasses dressing (direct application) (+3 mds. 38 srs., +4 mds. 9 srs., +3 mds. 35 srs. and +2 mds. 9 srs.). Plot F shows a definite depressant effect as compared with the control (—2 mds. 27 srs.). Castor cake treated plots show lower yields than concentrated manures.

TABLE XI

Calculations on the cane yield and total sucrose per acre—Co 312.

Treatments	Cane yield in 1/90th acre	Cane yield per acre	Sucrose per cent cane	Total sucrose per acre	Excess cane yield over control	Excess cane yield over F	Excess sugar yield over control	Excess sugar yield over F
	Mds.	Mds.		Mds.	Mds.	Mds.	Mds.	Mds.
A	11—26	1048·5	12·22	128·15	+104·75	+355·5	+17·70	+42·83
B	11—37	1073·25	11·09	118·96	+139·50	+380·25	+6·70	+33·60
C	11—23	1041·25	12·27	127·70	+107·50	+348·25	+16·30	+42·40
D	10—37	983·25	12·21	120·05	+49·50	+290·0	+8·62	+34·75
E	10—36	731·0	12·52	111·52	—200·0	+38·0	+0·09	+26·22
F	7—28	693·0	12·31	85·30	—240·75	...	—26·13	...
G	11—10	1012·5	11·62	117·65	+78·75	+319·5	+6·22	+32·35
H	10—23	951·75	10·56	100·50	+18·00	+258·75	+10·93	+15·20
I	10—15	933·75	12·47	111·43	...	+240·75	...	+26·13

The cane variety Co 312 of all the three varieties taken have responded most effectively to the concentrated manures. The depressant action of direct application of molasses has been evident not only in the case where molasses has been administered in heavy doses (treatment F) but also in the case of application in light doses (treatment E).

Taking the cane yield per acre while concentrated manure A, B, C, D treatments show an increase of 144·75, 139·5, 107·5 and 49·5 mds. respectively, molasses, direct application in light and heavy doses (treatments E and F), show a deficit of 200 mds. and 240·75 mds. respectively. It may be noted that castor cake in light and heavy doses (treatments G and H) do not respond so well as the concentrated manures (+78·75 and +18·0 mds. respectively over control). The excess of cane yield over F (plot heavily treated with molasses) with concentrates manures A, B, C, D are uniformly high (+385·5, +380·25, +348·25+290 mds. respectively). The castor cake plots G and H show an increase of 319·5 and 258·75 mds. while the control plot shows an increase of 240·75 mds. The plot receiving light dose of manure shows an increase of 38 maunds over F.

The increase in sugar yield per acre as compared with control in case of A, B, C and D are 17·7, 6·7, 16·3 and 8·62 mds. respectively, while E shows no increase (+0·09 mds.) and F indicates a deficit of 26·22 mds. Castor cake (G and H) treatments show an increase of 6·22 and 10·93 mds. respectively. The excess in sugar yield per acre over F in case of A, B, C and D are 42·83, 33·60, 42·4 and 32·74 mds., while castor cake (G and H) show an increase by 32·35 and 15·2 mds. respectively. Control indicates an increase of 26·13 mds. over F.

TABLE XII
Co 331, LATE-RIPENING VARIETY

Percentage of total sucrose in cane obtained under different manurial treatments (Tests carried out after verification of the maturity by top-bottom brix and purity ratios)

Treatments	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Average
A	11.12	11.14	11.49	10.64	11.74	11.20	11.22
B	11.36	9.71	11.39	11.47	10.99	11.31	11.04
C	11.67	10.29	12.11	11.42	12.16	12.26	11.65
D	11.44	10.45	11.90	11.02	11.44	11.65	11.32
E	11.89	10.76	11.88	11.09	11.44	12.58	11.61
F	11.11	8.99	11.22	11.59	9.66	11.38	10.66
G	11.00	10.73	11.53	11.40	11.67	11.12	11.24
H	11.29	10.53	11.38	11.41	12.29	10.78	11.28
I	11.10	10.35	11.71	11.23	11.01	12.1	11.25

In Co 331, a late-ripening variety, we find as we observed in other cases, the total sucrose in cane in all treatments tends to a maximum and is nearly the same irrespective of the manurial treatments. F, i.e. the plot receiving heavy molasses dressing, however, shows a definite decrease in sucrose content.

TABLE XIII
Yield of cane Co 331 per plot of 1/90th acre (Harvested on 1st March 1939)

Treatments	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Average of six replications
	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
A	9-5	10-7	8-20	9-7	8-15	8-2	8-36
B	8-28	9-30	10-8	8-37	8-31	9-28	9-14
C	9-7	8-15	9-21	10-5	8-28	9-15	9-29
D	8-37	8-36	7-36	7-20	10-33	9-1	8-34
E	9-3	10-0	9-10	11-18	9-6	9-30	9-31
F	6-38	7-27	7-5	8-38	6-28	6-12	7-10
G	8-30	9-1	9-30	9-26	9-0	9-12	9-11
H	7-30	10-10	9-30	8-26	9-11	9-26	9-9
I	8-32	9-30	8-20	9-8	9-2	9-18	9-5

TABLE XIV

Yield of cane and sucrose per acre and calculation of the excess of cane and sugar yields over control and F treatment

Treatments	Yield of cane per 1/90th acre	Yield of cane per acre	Sucrose per cent cane	Total sucrose per acre	Excess cane yield per acre over control	Excess cane yield per acre over F	Excess sugar yield per acre over control	Excess sugar yield per acre over F
	Mds.	Mds.		Mds.	Mds.	Mds.	Mds.	Mds.
A	8-36	801.0	11.22	89.98	-20.25	+146.25	-2.90	+20.19
B	9-14	841.5	11.04	101.51	+20.25	+186.75	+8.09	+31.72
C	9-29	875.25	11.65	101.96	+54.00	+220.50	+9.54	+32.17
D	8-34	796.5	11.32	90.16	-25.25	+141.75	-1.26	+20.39
E	9-31	879.75	11.61	106.5	+58.50	+225.00	+14.08	+36.71
F	7-11	654.75	10.66	69.79	-166.50	...	-22.63	...
G	9-10	832.5	11.24	93.57	+11.25	+177.75	+1.15	+23.78
H	9-9	880.25	11.28	99.29	+59.0	+225.50	+6.87	+29.50
I	9-5	821.25	11.25	92.42	...	+165.50		+22.63

In the case of Co 331, a late-ripening variety, the cane yields with concentrated manures as compared with the control plots have shown significant results with B and C treatments, the performance in the case of A and B being negative. The anomaly in the results is due to better cane yields in the control plot. Castor cake treated plots (G and H) have given good result and so has E having light dressing of molasses. The most outstanding result, as has been observed also with other cane varieties, is the depressant action of molasses in heavy doses, where there is a deficit of 166.5 mds. in the cane yield.

Comparing with treatment F the yield of cane is uniformly high with concentrated manures (+146.25, +186.25, +220.50 and +141.75 respectively). The performance in the case of castor cake treated plots is about the same as the prepared manures (+177.15 and 225.5 mds. respectively). The deleterious effect is not evident in case of light dressing of molasses, the increase in cane yield over control being quite high (+225 mds.). Even the cane yield in the control plot is greater than F by 165.5 mds.

The sugar yields are synchronous with the cane yields since the percentage of total sucrose is more or less the same irrespective of the manurial treatment.

Cane variety may be one of the factors determining the response to different manures. It may be said that whereas Co 313 and Co 312 respond quickly to the concentrated manures, it does not do so much with Co 313.

CONCLUSIONS

1. An easy biological process has been evolved for the conversion of fairly large quantity of exhaust cane molasses into a clean, dry inodorous manure with a high nitrogen content.

2. It requires installation of a small manure-making plant, as described in the paper in detail, requiring a few masonry tanks, centrifugal pumps, air blowers and leading pipes.

3. It was revealed that, whereas fermentation under acid condition without neutralization lead to loss of nitrogen, fermentation with intermittent addition of lime to neutralize the acidity gave definite evidence of nitrogen fixation being about twice the total nitrogen content in the original molasses.

4. It was found that the use of pure culture of yeast as started instead of mixed bacteria consisting of yeast, *B. coli*, acetic and lactic organisms gave sludge with higher nitrogen content than that obtained when mixed bacteria was used. The nitrogen fixation with molasses and filter press cake mixed in equal proportions using pure culture of yeast as starter gave nitrogen fixation about three times the total nitrogen present in the original molasses.

5. By a series of experiments taking a particular yeast variety where neutralization was effected with sodium carbonate solution instead of milk of lime it was found that heavy aeration led to about one-and-a-half times the yield obtained under normal conditions of fermentation. The use of ammonium sulphate, and more significantly ammonium phosphate, to the extent of 1-5 per cent gave increased yeast yield (three times using ammonium sulphate and six times using ammonium phosphate). Higher yeast yields were obtained on fermenting at increasing dilutions, sp. gr. 1017 giving the highest yield.

6. Thus fermentation at the neutral point, i.e. under conditions where the acidity is constantly neutralized by milk of lime led to increased yield of yeast.

7. The biological process evolved gives product like yeast, calcium acetate, propionate, lactate, butyrate and also nitrogenous decomposition products, as indol and skatol acetic, propionic, butyric acids and their esters which are said to contain plant hormones are likely to be present. Thus whereas molasses has very little value as manure, its biological products contain valuable plant food. The biological process of conversion of molasses into manure augments the yield of yeast, calcium salts of organic acids and also the plant hormones described above. An investigation into the existence of the hormones by micro-methods is in progress. It is argued that fermentation with aeration and constant neutralization increases the yeast yield and diverts the course of fermentation so as to give the maximum production of yeast with least alcohol formation. The latter point is also being investigated.

8. Cropping tests were conducted with two manures prepared by the rapid process—molasses manure with intermittent addition of lime with aeration and molasses and filter press cake manure at the Kalyanpur Farm, United Provinces, in collaboration with the Agricultural Department. Working with three varieties of cane, Co 313, Co 312 and Co 331—early, medium and late ripening varieties—with six replications in randomised blocks indicate that molasses, when directly applied in the soil, had definite depressant effect as compared with the control in heavy doses, which increased with increased application although in smaller doses the effect was not very significant. In the case of Co 312, depressant effect was evident when molasses was applied both in light and heavy doses.

The concentrated manures gave definitely increased cane yields comparable with yields obtained by using castor cake as manure. The sucrose per cent in cane was slightly higher with concentrated manures than with

molasses. Taking a particular variety, Co 313, the yield of cane was approximately 100 mds. more per acre with the prepared manures as compared with the control, while molasses direct application gave 100 mds. cane less. There was an increase of about 20 mds. of sugar per acre with the new manures over the control, while there was a decrease in the yield of 20 mds. of sugar in case of direct molasses application.

9. Direct molasses application lead invariably to diminished percentage of germination and disease, such as yellowing of leaves, white ants, etc., while the prepared manures made canes less susceptible to such attacks.

10. The cost of production of the manures come to approximately 12 annas per maund giving a return of the cost of molasses to sugar factories at 4 annas.

11. Considering the relative efficiencies 20 mds. of molasses will have the same effect as 3 to 4 mds. of the concentrated manures.

ACKNOWLEDGEMENTS

My thanks are due to Mr. R. C. Srivastava, B.Sc., O.B.E., Director, Imperial Institute of Sugar Technology for his valuable advice and giving me the necessary facilities for carrying on the work.

I have particularly to thank Mr. P. B. Richards, I.A.S., Director of Agriculture, United Provinces, for taking a keen interest in the concentrated manures prepared by the biological process and placing at my disposal three acres of land and the necessary staff for arranging for the cropping trials at the Kalyanpur Farm, United Provinces. The name of Mr. Gajadhar Lal, B.Ag., Superintendent of the said farm is also to be particularly mentioned for careful planning of the plots and recording various details regarding cropping tests.

I have also to thank Mr. J. P. Shukla, M.Sc., my assistant for furnishing me with the harvesting data and analysis of cane samples and Mr. G. S. Datta, B.Sc., for preparing the manures and providing me with some data regarding nitrogen balance under various conditions of manufacture of manures.

REFERENCES

- Bhaskaran, T. R. and Subramanyan, V. (1937). *Proc. Sugar Tech. Assoc. India, Sixth Convention*, p. 424-32
- Desai, S. V. and Fazul-ud-Din (1938). *Ind. J. Agric. Sci.* **8**, 38
- Gainy (1923). *J. Agric. Res.* **24**, 289-96
- Kogl (1937). *Nature* **140**, No. 3544
- Narayana (1932). *Symposium on the Role of Organic Matter in Soil. Soc. of Biol. Chemist India*, p. 14
- (1937). *Proc. Sugar Tech. Assoc.*
- Paramanik, B. N., Rao, R. B. and Lal, J. B. (1937). *Proc. Sugar Tech. Assoc. India, Sixth Convention*, p. 188-96
- Sen, H. D. (1938). *Indus. Ind.*, p. 250-1
- Sen, H. D. and Dutta, G. S. (1937). *Proc. Sugar Tech. Assoc. Sixth Convention*, p. 333-43
- (1938). *J. Sci. & Tech. Ind.* **4** (1 and 2), 59-63
- Tinkler (1937). *Nature* **140**, No. 3544
- Viswanath and Suranarayana (1932). *Symposium on the Role of Organic Matter in Soil. Soc. Biol. Chem., India*, p. 8

A REVIEW OF THE APPLICATION OF STATISTICAL THEORY TO AGRICULTURAL FIELD EXPERIMENTS IN INDIA*

BY

P. C. MAHALANOBIS

Statistical Laboratory, Presidency College, Calcutta

I. GENERAL REVIEW OF THE PRESENT POSITION

A REVIEW of the application of statistical theory to agricultural field trials in India in recent years is largely a story of the triumph of methods devised by R. A. Fisher at the Rothamsted Experimental Station. The new developments also bear remarkable testimony to the scientific vision of Sir John Russell, Director of the Rothamsted Experimental Station, who had recognized as early as 1919 the need of the application of statistical theory to agricultural research, and had not only persuaded Fisher to take up this subject but had given him full scope and freedom for working out appropriate statistical methods in his own way.

The basic principles of the new method are now well known and need not be discussed in detail. In order to appreciate the revolutionary advance brought about by the introduction of the new technique, let us however consider for a moment the contrast between experiments of the old and new type.

The old type of field experiment

Suppose we wish to compare the yield of say six varieties or the effect on yield of six kinds of manures. In the old type of experiment the field would be divided into six plots, and a single plot would be allotted to each treatment. As Fisher [1935] explains 'the treatment giving the highest yield would of course appear to be best, but no one could say whether the plot would not in fact have yielded as well under some or all of the other treatments'. It is known that within the same field wide differences exist in the fertility of the soil. Even when the soil fertility is uniform, there are innumerable other causes which affect the yield. How can we be sure that the observed differences in yield are due to the difference in the treatments, and not to soil heterogeneity? How can we be sure that they are not due to chance fluctuations? This is the basic problem. In order to solve it we must eliminate the effect of soil heterogeneity, and make an unbiased estimate of the magnitude of errors due to chance so that we may be sure that the observed effect is significant in comparison with the size of such chance errors.

The Fisherian technique

Let us now see how Fisher solved the problem. Consider the same experimental field which had been originally divided into six portions.

* Presented before the meeting of the Board of Agriculture and Animal Husbandry in India held in Lahore in December 1937

Fisher simply further sub-divided each portion* into a number of plots of smaller size ; and within each portion (or block as he called it) he assigned one plot to each treatment but strictly in a random manner. We have now the randomized block in its modern form. Using the principle of block division in two directions symmetrically we get the well-known Latin square.

Results governed by laws of chance

The important point to be noticed is that the results will be now governed entirely by the laws of chance. There are innumerable causes which produce differences between the plots, and we know from the conditions of the experiment that it is impossible in practice to secure that the plots will be all alike. But the validity of the estimate of error is now guaranteed by the process of randomization, namely 'the provision that any two plots, not in the same block, shall have the same probability of being treated alike, and the same probability of being treated differently in each of the ways in which this is possible' [Fisher, 1935]. The calculus of probability and the apparatus of the statistical theory of sampling distribution can now be used with complete confidence. The logical foundations of scientific inference were thus made secure, and agricultural experiments were placed for the first time on the same footing as experiments in other sciences. In actual fact the statistical theory of exact distribution in terms only of actual observations, popularly known as distributions in 'Studentized form', achieved a good deal more. It made possible general conclusions being drawn with logical rigour from particular observations. But this is a topic of statistical rather than agricultural interest and must be passed over here.

Elimination of soil differences

The second point to be observed is that by the technique of block division the problem of soil heterogeneity was solved at the same time. As each block contains all the treatments once and once only, differences between the total yields of the different blocks could safely be ascribed, apart from errors of sampling, to soil differences ; and could be eliminated by suitable statistical methods. This of course led to a great improvement in the precision of the comparisons. When we remember that in particular experiments in India as much as 90 per cent of the total variation is sometimes caused by soil differences, the importance of eliminating its effect will be easily appreciated.

The analysis of variance

The third point to be emphasized is the close connexion between the field procedure and the procedure of statistical analysis in the Fisherian technique. In fact they are merely two aspects of the same problem ; and to quote Fisher [1933] 'once the practical field procedure was fixed, only a single method of statistical analysis could be valid. . . The specification of the particular process of randomization carried out, determined in advance the correct statistical analysis of the results'.

*I need scarcely add that the experimental field may be divided into any number of convenient portions each of which is further sub-divided into a number of plots.

To sum up then, replication, randomization, and block division (or local control) were the principles of design introduced by Fisher [1923] at Rothamsted. Replication is essential because it is the sole source of the estimate of error, while randomization is necessary to guarantee the validity of the estimate, i.e. to ensure that the estimate will be unbiased. The purpose of block division is to increase the precision of the comparisons by elimination of soil differences, while replication is also useful in securing the same object by diminishing the experimental error. Finally the analysis of variance* gives a convenient and valid method of extracting the information contained in the observations. As Wishart has pointed out, the Fisherian technique 'was something in the nature of a revolution,' and altered the subsequent course of agricultural experiments throughout the world.

Previous conditions in India

It took some time before the new technique was introduced into this country. Seven or eight years ago in India the control used to be almost always repeated, but the treatments were usually laid down without replication. Even when replication was used, it was of the systematic type and inadequate in number. In interpreting the results, the usual practice was to compare the means of the various treatments. In a few cases probable errors of means were calculated. The ordinary formula in the classical theory of errors was used for this purpose. This was inexact for the twofold reason that the observed variance was substituted for the corresponding population value, and the effect of using small samples was ignored.† Besides in the absence of randomization, such estimates were not unbiased, and could not be validly used for purposes of comparisons. Finally there was no attempt to eliminate the effect of soil differences. It is no wonder therefore that many of the inferences drawn from the old experiments were unreliable. Even when the results were true, this could not be asserted with scientific precision. A fair idea of these old-type experiments, which used to be conducted in India a few years back, can be obtained from 'Analysis of Manurial Experiments in India' by Vaidyanathan [1934].

Introduction of the new technique in India

Like most other recent movements in agriculture in India, we owe the introduction of statistical methods to the Royal Commission on Agriculture (report, pages 617-8), which had made definite recommendations on this point in 1928. In actual practice the modern period of field experiments began in India, I believe, with the foundation of the Imperial Council of Agricultural Research in 1929 on the recommendation of the Royal Commission.

* See note on 'variance', 'standard error', 'covariance', etc. (Appendix II)

† It is of some personal interest to me to recall here that to this particular problem I owe my contact with agricultural work. In 1924 my attention was drawn by Dr. W. Burns (Agricultural Commissioner with the Government of India), then working in Bombay, to an experiment in which six varieties of rice were laid out in ten replicates systematically arranged side by side in long stripes. On the assumption of a systematic variation in soil fertility, it was possible to eliminate the soil differences by graduation, and it was found that the precision of the comparisons could be considerably increased. At that time I was quite unfamiliar with the Rothamsted work, but Dr. Burns' problem soon made me get acquainted with the Fisherian method, and made me realize its great value.

The earliest experiment of the new type, a varietal trial on rice with a 12×12 Latin square was reported in the *Indian Journal of Agricultural Science* in 1931. The Imperial Council of Agricultural Research from its inception laid emphasis on statistical methods, created a statistical section at headquarters with a whole-time statistician at its head, and gave a grant to the Statistical Laboratory, Calcutta, for advanced studies and researches in statistics. In fact I believe it was soon made a condition of all Imperial Council of Agricultural Research schemes that the experimental designs should be of the approved type. The Statistician to the Imperial Council of Agricultural Research gives his advice on all standard schemes in the province, and personally visits a large number of farms every year. Help is also available, especially on the research side, from the Calcutta Statistical Laboratory. In the course of this work a series of 'Statistical Notes for Agricultural Workers' was started of which 24 numbers have been published so far. In 1932 arrangements were made in Calcutta for giving special courses of instruction in statistical methods to officers who were sent there on deputation for this purpose. During the last five years such training has been given to over 75 agricultural officers from all over India, which, I believe, has helped materially in raising the general standard of work. The lead given by the Imperial Council of Agricultural Research in all these ways has resulted in the Latin square and randomized block designs being used with great success all over India. It is probably no exaggeration to say that no important experiment in India is now laid out on an old type design. This must be considered to be a solid achievement.

Factorial (complex) experiments

We may now consider some further developments of the new techniques. As early as 1926 Fisher had advocated the use of factorial designs in which two or more types of treatments were laid out on the same field.

Suppose we wish to compare three varieties, and the effect of three manurial treatments on each of these varieties. If we conduct the experiments separately, and use six replications, we shall require for the varietal trial $3 \times 6 = 18$ plots. For the manurial portion we shall require three experiments, dealing respectively with the three varieties. With six replications we shall therefore require 54 plots for the manurial investigations and 18 plots for the varietal comparison or 72 plots altogether.

Instead of simple experiments, suppose we combine them in one factorial (or complex) design. First of all, for nine combinations (3 varieties $\times 3$ manures) we can then afford to give eight (instead of six) replications each in the same field of 72 plots. Secondly, we shall have no less than 24 replications available for the varietal or manurial comparisons; so that, if the standard error per plot remains the same, the accuracy of the main comparisons will be increased four times. Finally, the three manurial treatments cannot be directly compared in the separate experiments; but in the factorial design the comparisons would be completely valid. In other words, the differential manurial requirement of particular varieties, i.e. the interaction between varieties and manures, if any, can be investigated only if the experiment is designed in the factorial form. With three or four factors the amount of information obtained is proportionately even greater. Besides the main effects,

we can not only study the differential effect (or interaction) of the factors two by two, but also the response of one factor in the presence or absence of two or more of the other factors.

A factorial experiment is thus not only more efficient in the sense that with the same number of plots all the factors can be studied with greater precision, but is also more comprehensive and will give information about differential response which could not possibly have been obtained by any number of experiments of the simple type. This is why Fisher [1926] definitely rejected the orthodox principle of varying the factors only one at a time and said : 'No aphorism is more frequently repeated in connexion with field trials than that we must ask Nature few questions, or ideally, one question at a time. The writer is convinced that this view is wholly mistaken. Nature, he suggests, will best respond to a logical and carefully thought out questionnaire ; indeed, if we ask her a single question, she will often refuse to answer until some other topic is answered.'

Before leaving this topic it is perhaps worth while pointing out a third advantage of the factorial design. In the orthodox method all the factors except one are deliberately kept approximately constant. In the result, information is obtained only for a narrow range of controlled conditions. In the factorial design on the other hand a number of factors are allowed to vary at the same time, so that conclusions drawn from such an experiment have a much wider basis for induction.

In India the first factorial experiment with three varieties of potato under three manurial treatments was laid down at the instance of the Statistical Laboratory at the Visvabharati Institute of Rural Reconstruction at Sriniketan in 1931. During the last four or five years similar two-factor experiments have become quite common all over India. Designs with three or four factors are also being used with success. As an example I may mention the four-factor cultivation experiment with rice (three varieties, five dates of planting, three spacings, and three numbers of seedlings per hole) designed at the Statistical Laboratory and conducted under the Imperial Council of Agricultural Research rice research scheme at Chinsurah for the four seasons 1933-37. The summary of results shown in Appendix I will give some idea of the wealth of information which can be obtained only from designs of this type.

In spite of their efficiency and comprehensiveness certain objections have been raised against the use of factorial designs which may be briefly considered here. It has been pointed out that the main effects are obtained with greater precision than the interactions ; also that the experiment includes many combinations which are never likely to be used in practice. This is quite true but inevitable. When we have no knowledge as to what particular combinations are likely to be useful, it is desirable that we should seek to survey the whole range of all the factors. But an extensive field of survey inevitably implies a lower level of accuracy. However as experience is gathered, the field of enquiry can be narrowed by reducing the number of combinations, with an automatic increase in the precision.

A second objection is more serious. With an increase in the number of combinations, the size of the block becomes too large for adequate elimination of soil-heterogeneity with consequent increase in the residual error. The

difficulty has been admirably got over recently by the 'splitting of plots' and the 'confounding of interactions'.

Split-plot and 'confounded' designs

In the factorial design complete information about all the combinations can be obtained at the cost of accuracy. We can, however, increase the precision by sacrificing a portion of the information. This is just what is achieved in the 'confounded' design. The whole array of treatment combinations is therefore not included in the same block, but deliberately distributed over two or more balanced sub-blocks. Experience has shown that high-order interactions are often insignificant, or even when statistically significant are not of much practical importance. In the confounded design information about such high-order interactions is usually sacrificed to increase the precision of other comparisons. If we like we can, however, arrange to obtain some information about all the interactions, but inevitably at a lower level of precision, by 'partial confounding'.

The split-plot lay-out is a simple example of confounding in which the main effects of one of the factors are confounded. This design is particularly useful when some of the treatments are such that they cannot be conveniently applied to small plots. These main treatments are therefore laid out in a randomized block or Latin square design, but each whole-plot is divided into a number of sub-plots which are allotted at random to the different sub-treatments. The residual variance between sub-plots gives the appropriate error for the comparison of sub-treatments, while the residual variance between plots gives the error for the whole-plot treatments.

The split-plot design is being extensively used in India, but the confounded design has so far not attracted much notice.* As far as I know, one elegant design prepared by Yates has been laid down at the Tocklai Tea Experimental Station, and one design has been supplied by the Statistical Laboratory to the Dacca University for the Imperial Council of Agricultural Research scheme.

The designing of confounded lay-outs is an interesting exercise, and in skilled hands it has attained a high degree of efficiency. I would draw the attention of all agricultural workers interested in this subject to the discussion by Fisher [1935], Yates [1937] and that in the *Rothamsted Reports* for the last few years.

Complete factorial designs, as we have seen, are both efficient and comprehensive. But they need great care at every stage of the work, and with a large number of factors require blocks which are inconveniently large in practice. There is, therefore, a limit to the usefulness of this type of design depending on the heterogeneity of the land, the number of factors and nature of the problem, the skill and experience of the investigator, etc. The split-plot design is very convenient in problems in which knowledge about the main treatments is already available. But I am of opinion that it is the confounded design which has the greatest possibilities in India, both on account of its flexibility as well as its economy of cost. Caution is needed, however, both

* This review was originally written in December 1937. Since then the principle of confounding is being increasingly used in India.

in designing the experiment and in carrying out the statistical analysis. In the beginning, therefore, it will be desirable to use standard patterns under the guidance of statistical workers.

Interpretation of results

Before leaving this subject I would like to add a few words in regard to the interpretation of the results. I have found that many agricultural workers are able to reduce the data correctly and complete the arithmetical part of the analysis of variance without, however, being able to draw the necessary inferences. 'Significance' and 'non-significance' are purely technical terms with the exact implication of which every experimenter should be familiar.

Suppose we are working on the five per cent level of significance. Then the rule is that any effect which is likely to occur by pure chance less than once in twenty times on an average will be called 'significant'. On the other hand, effects which are likely to occur more frequently than once in twenty trials will be called 'non-significant'. Let us see the application of this rule in a concrete case. Suppose we have an experiment in which the treatments do not in fact produce any effect. Even then, with the present rule, the effect will appear to be significant about once in twenty trials, and in the remaining 95 per cent of cases we shall quite correctly decide the effect to be nil. The risk of considering an effect to be real, when in fact it does not exist is thus limited to just five per cent. Similarly working at one per cent level of significance, we limit the risk of our accepting a spurious effect as real to one per cent. In other words we work with odds of 99 to 1 in our favour.

I may point out at this stage a peculiar property of statistical inference. Suppose we are working on five per cent level. We have seen that even when the effect is nil, we shall judge it to be real once in 20 trials. In other words, if statistical theory is right, we must be wrong in our judgment in five per cent of the cases. The possibility, or rather, the certainty of error is thus inherent in the structure of statistical inference. This knowledge is a salutary check against an exaggerated sense of our own infallibility.

The experimenter must, therefore, be careful in attaching undue importance to an isolated result which may appear to be statistically significant and yet does not fit in with general agricultural experience. Such results should not be ignored, but should neither be accepted until corroborated by further experiments. On the other hand, results statistically insignificant should not be always neglected. If they appear to be plausible from other considerations, further investigations should be made with increased precision of comparison.

In short, the experimenter must use his critical judgment and discretion in the final interpretation of the results. Statistics is both indispensable and invaluable, but it cannot replace the human mind.

Precision of Indian experiments

Having reviewed the broader features of the new technique, it will be of some interest to examine the precision attained in Indian experiments. I am sorry, in the limited time at my disposal, I was unable to collect relevant information from the different provinces of India. I shall, therefore, discuss

this point with the help of materials from Bengal and Assam which were readily available in our Laboratory.

The average standard errors per plot (expressed as percentages of mean yields) for four or five careful series of varietal trials with *aus* and *aman* rice at Chinsurah Farm during the five seasons 1932-33 to 1936-37 are shown below. (The figures within brackets give the number of experiments on which the average is based.)

Bengal : Chinsurah Farm varietal tests
(Standard error per plot as percentage of mean)

Year.	Rice crop	
	<i>Aus</i>	<i>Aman</i>
1932-33	11.61 (3)	10.10 (5)
1933-34	9.68 (2)	10.06 (5)
1934-35	8.38 (3)	10.21 (6)
1935-36	..	19.10 (5)
1936-37	9.19 (2)	9.74 (5)

Similar figures for recent rice and sugarcane experiments in Assam for the period 1932-33 to 1935-36 are given below.

Assam
(Standard error per plot as percentage of mean)

Centre	Crop	Variety	Manure	Complex
Karimganj	Rice	6.78 (24)	8.00 (4)	8.25 (6)
Titabar	Rice	10.50 (20)	7.98 (4)	..
Jorhat	Sugarcane	8.21 (9)	7.71 (7)	15.40 (4)

It will be noticed that in Bengal and Assam in the case of rice and sugarcane, a standard error of 8 or 10 per cent per plot is quite usual.

Comparative figures for English experiments are quoted below from the Report of the Rothamsted Experimental Station for 1935.

English stations

Crop	Latin square	Randomized block	All arrangements
Potato	6.8	9.2	..
Sugarbeet	6.1	7.9	..
Swedes	6.9
Mangolds	8.2
Kale	7.7

Wishart and Sanders [1936], are of opinion that a standard error of 5 per cent for root crops and of 10 per cent for cereals may be considered satisfactory. Judged by English standards, the work in Assam and Bengal is therefore not unsatisfactory. I have no reason to think that careful work in other parts of India is in any way less accurate.

Latin square v. randomized block

Owing to the possibility of eliminating soil differences in two directions, one would expect the Latin square to be more accurate than the randomized block, and English experience has generally borne this out. I am not in possession of enough data to judge the position in India. My general impression is that the Latin square has been given preference here in small-scale varietal work. For large-scale work, I think on the whole the randomized block has been used more extensively in India, no doubt on account of its greater flexibility. One advantage of the randomized block is that an estimate of error can be calculated separately for each comparison. Yates [1935] has pointed out that 'this is of great value when handling new and unknown material, or treatments which may produce large differences and even partial or complete failures. In such cases the assumption of constancy of error variance is entirely unjustified, but in a randomized block experiment any treatment or treatments may be excluded and the analysis carried out on the remainder. This is not true of either the Latin square or of confounded arrangements'.

Complex or factorial designs in India apparently have a slightly higher standard error per plot (of the order of 10 or 15 per cent of mean yield) than the simple Latin square or randomized block. This is probably due to the experimental difficulties in managing more than one set of factors on the same plot and to large block sizes.

Uniformity trials

In a randomized block design the greater the homogeneity of plots within blocks the greater the accuracy of the experiment. In practice this can be secured experimentally only to a limited extent. But sometimes it is possible to increase the precision of comparison very considerably by suitable statistical adjustments. Suppose, for example, that the initial fertility of plots is known from a previous uniformity trial in which the same variety is planted on all the plots and given the same manuring, and the relative fertility of individual plots remains fairly stable; then the yields in a succeeding year will be appreciably correlated with the yields in the uniformity trial. In this situation it is possible, with the help of the analysis of covariance, to make allowances for the initial differences in fertility among plots within each block. The use of such adjusted yields can then be used for the final comparison. This method has been sometimes known to have increased the precision even ten or twelve times.

It should not be imagined, however, that this is always or even generally possible. In fact with annual crops the fluctuations in fertility of the same plot from year to year are usually so great that the increase in precision obtained by this method is not in general commensurate with the expense or the delay of one season involved in a uniformity trial.

It is therefore usually unprofitable to conduct a uniformity trial as a preliminary to the main experiment with a view to increasing its precision. Repeating the actual experiment twice would most often give more information. This is why we have for a long time discouraged the adoption of a uniformity trial as a routine practice. It may be noted, however, that there are special circumstances in which such trials may be very useful indeed, for example, in the case of horticultural experiments.

Size and shape of plots

We may now consider the question of size and shape of plots. As early as 1910, Hall harvested a wheat and a mangold field in small units, and found that the variation between plots was appreciably reduced until the size reached was about 1/40 acre. It was therefore concluded, and the conclusion was corroborated by other uniformity trials, that the optimum size in England was somewhere about plots of 1/40 acre.

We have had occasion to examine the results of a number of uniformity trials in India, and we found that for varietal trials in many cases the plot could be reduced, so far as precision is concerned, to a very small size of the order of 1/140 acre. Plots of size 1/80 acre or 1/40 acre also give quite good results and can be safely recommended for convenience of agricultural operations. Given the area, the question of shape or orientation comes in. Christidis [1931] showed from theoretical considerations as well as experimental data that long plots placed parallel to the fertility gradient gave the best results. Our experience in India is also more or less similar. Sugarcane experiments at Pusa and other places in North Bihar show that strips, the length of which is 10 or 15 times greater than the width, give more accurate results. Rice experiments show the same tendency but to a smaller extent.

Size of blocks

The final precision of an experiment does not depend only on the best selection of plot size. What is needed is a choice of the optimum combination of the size of both blocks and plots. The best results will be obtained when the blocks are fairly homogeneous, (i.e. all the plots within the same block have nearly the same fertility), but differ appreciably as a whole between themselves. It is obviously not possible to give any limits for the block size. If the soil is fairly uniform, it is possible to work with blocks of a large size; on the other hand, if the fertility gradients are steep, the size of the blocks must be kept small. I had the opportunity of studying in detail the variation in soil fertility of a field of about one acre under rice at the Chinsurah Farm, which was harvested at my request in 7040 units of 9 inches by 90 inches (1/7744 acre). We tried many combinations of block and plot sizes, and found that a low standard error of about 3.5 per cent of mean yield per plot was obtained with a block size of 80 ft. \times 44 ft. (about 1/12 acre) with 8 plots each of size 20 ft. \times 22 ft. (1/100 acre). But considerably larger size of blocks 160 ft. \times 44 ft. (or about 1/6 acre), or 160 ft. \times 88 ft. (1/3 acre) with 8 or 16 plots each could be used with only a moderate increase in the error to about 5 per cent of mean yield per plot.

Success of the new technique

From the brief review given above, I think it can be stated without hesitation that in India wherever the Fisherian technique has been used on proper lines in field trials, it has been found entirely satisfactory in every way and has given excellent results. The working procedure is very flexible so that it can be adapted to suit the most diverse problems and conditions of work.

A good deal of valuable information regarding soil differences and the relative accuracy of different types of experimental designs is also fast accumulating in India. It is desirable in designing a new experiment that each experimenter should utilize all available information relating to his own work. In this way he would be often able to get a good idea of the type of design likely to give the best results, and also to safeguard himself against too large a margin of error by using an adequate number of replications or other methods of controlling soil differences.

Concomitant variations and correlational analysis

I have already considered the use of uniformity trials, and I may now briefly refer to certain other methods of increasing the precision of field trials by using concomitant measurements and the analysis of covariance. The underlying principle is simple. In a field trial there are many other factors besides yield which can be studied, and it often happens that some of these factors are correlated with the yield in the sense that variations in such factors cause (or are associated with) variations in the yield. It then becomes possible to separate and eliminate that portion of the variation in the yield which may be ascribed to these factors. In this way the precision of the experiment can be often increased very considerably. For example, it may happen in a field trial that the yields of different plots are disturbed by variations in the number of plants which have established themselves. When such disturbances are due to causes which have no connexion with the treatments under trial, it is clear that there can be no objection to making allowances for such variations. In the present example, by counting the number of plants in the different plots we can easily eliminate the variations in yield due to variations in plant number, and hence increase the precision of the experiment.

Similar methods may be used for eliminating the influence of varying intensity of attacks of pests and insects in different plots. It would be most undesirable to reject some of the yields simply because they appear to be too low. As Wishart and Sanders [1936] have remarked, 'once a start is made in rejecting actual figures, there is no knowing where to stop . . . a little skill in the game will lead to very significant, but quite untrustworthy results. There is no wish to impugn the reader's honesty, but no man is so virtuous that he can afford to treat temptation with disdain'. The position would be quite different if some observations were recorded on the intensity of the insect attack in the different plots before the crop is harvested. Such records can then be used for making adjustments without bias.

The method of correlation or analysis of covariance can also be used with great advantage in other ways. If records of growth of the plant (height,

girth, tillering, etc.), are kept at different stages, such records can be correlated with the final yield, and may be utilized to furnish valuable information on many points. These methods deserve greater attention than they have received so far in India.

Missing yields of plots

Before leaving the subject of field trials I may refer briefly to another question which occasionally arises in practice. Owing to accidents or negligence on the part of the subordinate staff, it sometimes happens that the yields of one or more plots are missing or get mixed up. In such cases it is often possible to reconstruct approximately the missing yields by purely statistical methods, and thus recover much of the information which would have been otherwise thrown away. Formulæ for certain simple cases were given for this purpose originally by Allan and Wishart [1930] and a general solution was subsequently given by Yates [1933]. Additions to the theory have been made in the Statistical Laboratory and have been used with success for certain types of mistakes which had actually occurred in India. It cannot, however, be emphasized too much that such procedures are at best make-shift arrangements, and the damage done by careless work cannot be repaired by such methods. In any case these methods must be used with very great caution.

Use of random samples

The use of concomitant measurements usually involves a great deal of labour, which can be often reduced very considerably by adopting the method of random sampling. Consider an ordinary field trial. Suppose for any reason, such as shortage of labour or inclement weather or some other difficulty, it is found impracticable to measure the complete yield of each plot. In this situation we may take one or more random samples from each plot and measure the yield of these samples. Or consider the measurement of the height of plants at different stages of growth in the case of a field trial. For ordinary crops the number of individual plants in each plot is very large, and it is practically impossible to measure separately every plant in each plot. We may here take a random sample of the same number of plants in each plot, and measure only those plants which are included in these random samples. Sometimes complete enumeration is not only impracticable but even theoretically impossible. For example, if the dry weight of plants is sought to be studied at different stages of growth under different treatments, it is obvious that necessary measurements cannot possibly be carried on the same plants, but only on portions of the material under observation. In such situations there is no alternative but to have recourse to random sampling.

Fortunately, when used judiciously, this method is quite efficient, and the additional error introduced by this method is usually small. Thus, for instance, when the yields in a field trial are obtained by random sampling, the effective error variance will be simply the sum of the variance between plots and the variance, due to sampling. The latter being considerably smaller than the former, the increase in the variance will consequently be

small. The method of random sampling* has great possibilities which should be more fully explored in India.

Other applications of statistical theory

I have considered field trials at some length as this is the main topic for discussion. But statistical methods have also been used with success in other types of work in this country some of which may be briefly mentioned at this stage.

The principle of randomized replication has been used in pot culture, animal nutrition studies, experiments on incidence of pests, horticultural experiments, etc. Very recently it has also been used in silvicultural experiments at the Forest Research Institute at Dehra Dun.

Correlational analysis has been used in a number of investigations on the influence of rainfall and other weather conditions on crop out-turn, and although valuable results have been obtained, the scope of such studies has been unfortunately much restricted in India by the paucity of reliable crop data extending over a large number of years. A good deal of valuable work is being done, chiefly under the auspices of the Meteorological Department, in agricultural meteorology in which statistical methods are being used extensively. Modern statistical methods are being increasingly applied in linkage and genetic studies at the Indore Institute of Plant Industry and elsewhere.

More advanced statistical technique has been occasionally used for the investigation of special problems, such as detailed studies of frequency distributions of cotton fibre in the Cotton Technological Laboratory at Matunga; the use of composite tests of significance in plant physiological work; the use of quantitative measures of group divergence for the scientific classification of varieties, etc. In the limited times at our disposal it will not be possible to discuss with profit such recent developments in detail.

On the whole, it may be said that agricultural workers in India have shown great readiness in using statistical methods, and have fully responded to the lead given by the Imperial Council of Agricultural Research in this matter. Given necessary guidance and facilities, there is every reason to hope that the use of such methods will steadily extend in India.

II. THE FUTURE PROGRAMME

Problems of special importance to India

We may now consider the future programme. It was only natural that in the pioneer stage, much of the work in India followed closely the agricultural practice at Rothamsted and other English stations. But with the valuable background of experience gained during the last six or seven years, and with

* To be quite pedantic it should be called 'random sampling from random samples'. For, all statistical work is necessarily based on random samples. The plot yields in a field trial, for example, are considered to be random samples from the hypothetical population of similar yields from the same plots under similar treatments in similar circumstances. Complete enumeration here merely means measurement of complete yields of all the plots which together constitute one single random sample. In the method of 'random samples', smaller random samples are taken from the plots.

the better organization of statistics in India, the time has come for using statistical theory and developing suitable methods for the study of problems of special interest to our country. A few suggestions in this connexion may be useful as a basis for discussion.

Rainfall and irrigation in relation to agriculture

We are all familiar with the essential facts. Agriculture is the basic occupation, and the prosperity of trade, commerce, and industry are more dependent on it in India than in most countries of the world. The seasonal rainfall is concentrated within a comparatively short period, but fluctuates widely both in total amount as well as in distribution from year to year. A good monsoon with well-distributed rain usually means good crops and general prosperity, while a bad monsoon still causes, over a vast area, failure of crops and widespread distress.

Water conservation, irrigation, and drainage naturally constitute a subject of overwhelming importance, and I hope to be excused if I dwell at some length on this question. I have had the opportunity of studying in some detail the problem of rainfall and floods in Bengal and in Orissa. This has made me realize how great is its direct and indirect bearing on agriculture. In most parts of India, we have enough rainfall to produce sufficient foodstuff for our present population. Our real problem is to conserve the water, prevent waste, distribute the available supply in the most efficient way over different areas and at different times according to agricultural requirements for the production of the optimum crop, and finally to drain away the excess without causing any mischief. Viewed in this way irrigation, drainage, flood control, and agriculture are merely different aspects of the same fundamental problem.

We possess fairly satisfactory data about rainfall, owing to the activities of an efficient Meteorological Department. We also have some, though neither enough nor quite reliable, data relating to rivers. But unfortunately the chief gap is in the agricultural knowledge.

Let me give a concrete example. I had occasion recently to examine a large irrigation scheme in Bengal which had the dual purpose of supplying water for crops at times of deficient rainfall, and of flood flushing the area as an anti-malarial measure. The future health, prosperity, and happiness of one million people depended on the success or failure of the scheme. We could estimate from past records with reasonable accuracy what deficiencies in rainfall were to be expected in future. We could also calculate how much water could be supplied from the Damodar river at different parts of the season. But unfortunately the agriculturists were quite unable to supply reliable information regarding the optimum water requirement of paddy. It was not possible therefore to make any estimate with confidence of the increased yield which might be reasonably expected with irrigation from the available supply. And yet this was the critical factor on which everything hinged. If the increase in production was sufficiently large the scheme would succeed; otherwise it would fail. The effect of a wrong decision either way would be disastrous. If the scheme were abandoned when in fact it might have succeeded, a great opportunity would be lost. If it were proceeded with but failed, such failure would jeopardize for at least one generation the initiation of other

schemes even when the prospects of success were great. I had to make the best of a bad job, and tried to get round the difficulty by using statistical methods in a rather speculative fashion.

But that is a different story. To come back to our topic, we need then careful studies of the influence of rainfall and other climatic factors on crops. Such studies would be useful in two ways. First, for supplying badly needed information about the water requirement of crops. Secondly, for purposes of forecasting; even when a failure of crops cannot be prevented, early information may often enable ameliorative action being taken in time.

Permanent climatic series

It will be desirable therefore to start well-designed experiments in different parts of the country for studying the influence of rainfall and weather conditions on the yield of standard varieties of crops. The experiments will be definitely of the long-range type, and will be continued for many years. Arrangements will also be made for recording a number of carefully selected meteorological elements. In planning this series, the needs of the country as a whole will be naturally kept in mind, and the work will be standardized sufficiently to enable valid comparisons being made between results obtained at different stations.

Phenological observations

The question of starting systematic phenological observations (such as earliest and latest dates of flowering of well-known plants, passage of migratory birds, advent of seasonal pests and insects, etc.), may also be given careful consideration in the same connexion. Such observations are likely to prove useful in many ways, not only in the study of seasonal variations of the weather, but in the control of pests and blights, and in throwing light on the behaviour of plants to environmental conditions.

Irrigation experiments

Well-designed experiments will also have to be laid down for the direct study of water requirement and the growth of crops under irrigation. In the first stage it will probably be desirable to conduct such experiments under conditions in which both the supply and the drainage of water can be controlled at desired levels. As experience is gathered, it will no doubt be possible to approximate more closely to field conditions.

In certain parts of India waterlogging and floods are often of almost as great importance as the lack of water. Carefully designed experiments are therefore needed for studying questions of seepage, waterlogging, etc., under actual agricultural conditions.

Soil erosion is a problem of importance in many regions. This question is closely connected with run-off and drainage, and requires to be studied in relation to irrigation. The possibilities of using agricultural methods, such as planting of suitable crops of trees for controlling soil erosion, deserve investigation in the same connexion.

All these irrigation experiments, to give the best results, require the active cooperation of the engineer and the agriculturist; while the scope of using statistical methods is practically unlimited,

Soil studies

Another problem of great importance is the study of the soil, and of the changes in its condition, in different parts of the country. As regards progressive deterioration, the Royal Commission on Agriculture [1928] was of opinion : ' While paucity of records of crop out-turn throughout India over any long period of time makes the matter impossible of exact proof, we are of opinion that the strong presumption is that an overwhelming proportion of original lands of India long ago reached the condition to which experimental data point'.

Permanent manurials

Careful experiments are needed to study whether soil deterioration is still progressing, and if so at what rate, and also to study the influence of different types of manures to prevent such deterioration and maintain the soil in a healthy condition. The time has therefore come to lay down a series of permanent manurials on modern lines at a number of selected stations. Where practicable, the manurial series may be suitably combined with advantage with the climatic series.

Multiple experiments

Multiple experiments offer great advantages for the study of climatic, varietal, manurial, and other questions. In this plan a number of experiments of the same type would be laid down with the same or similar groups of varieties or treatments in different parts of the country. Owing to the large differences in soil and climatic factors, not only between different provinces but even in different districts of the same province, these experiments would be conducted under widely varying conditions.

The work will have to be planned as a whole. When the same set of varieties or manures or other treatments cannot be used in all the experiments of a given series, it should be still possible to link up the work by providing overlapping treatments through which comparisons can be made with confidence. Standardization will obviously be necessary, but sufficient flexibility must be retained to adapt the work to suit local needs.

If the multiple series is designed as a whole, it will be often possible to conduct a joint analysis of the results, and to study the influence of the variations in the different factors. In this way valuable information might be obtained in a few years which would otherwise take a very long time to collect. In 1931 I had pointed out the need and scope of such multiple experiments under Indian conditions and had pleaded for their adoption at the joint session of the Agriculture, Physics and Mathematics sections of the Indian Science Congress at Nagpur. Six or seven years ago the time was probably not ripe for undertaking such experiments on a large scale. But the Fisherian technique has now become so familiar that it should not be difficult to start them in the immediate future.

Cultivation and rotation experiments

Other problems of special importance in India are connected with methods of cultivation and rotation of crops. Given soil and water, the basic problem is to secure the greatest return to the cultivator. A wide outlook is

desirable in designing such experiments. When we compare different methods of cultivation, for example, it is obviously not sufficient merely to concentrate attention on which method gives the largest yield of crop. It is also necessary to take into consideration the question of relative costs, the real aim being to find out which method will secure the largest net return to the cultivator. Similarly, in rotation experiments it is not enough to concentrate attention on merely the influence of a particular crop in one year on the yield of another crop in a succeeding year. The object should be to find out that particular sequence of crops which, after making allowances for differences in the cost of cultivation, would on an average secure the highest profit over a number of years.

Crop-cutting experiments

Although crop-cutting experiments do not fall under field trials, I would like to point out how such experiments may be made to supplement the information obtained from field experiments. Consider any given region. In an adequately designed crop-cutting experiment this region will be divided into a number of homogeneous zones with more or less the same type of soil, climate, irrigation facilities, type of crop, method of cultivation, etc. Suppose we now arrange to conduct the crop-cutting work at a number of spots selected strictly at random (but so arranged as to include all the varieties or conditions we desire to study) within each zone. The experiment as a whole will then resemble, on a very large scale, a field trial with a design of the randomized block type. I am not suggesting that in practice it will be possible to preserve the analogy in detail. But I think it should not be difficult to plan a crop-cutting experiment as a whole in such a way as to supply useful information regarding the performance of different varieties or treatments under actual cultivating conditions on a large scale.

Apart from such considerations, a crop-cutting experiment of course has its very important primary function of supplying information about the total out-turn of crop. As the only method available here is that of random samples, this question offers great scope for the application of statistical theory. Valuable pioneer work has been done in the United Provinces in this connexion, but the subject is of sufficient importance to deserve systematic and sustained study in other provinces.

Place of statistics in agriculture

Before concluding I would like to make a few general remarks about the place of statistics in agriculture. It is I hope sufficiently clear from the previous discussion that the first function of statistical theory is to supply an adequate technique for collecting the primary data in such a way that valid inferences may be drawn from them. The use of the principle of randomized replication in some form or other is indispensable for this purpose. The second function is to extract the whole of the information contained in the data in the most efficient way. It has been already pointed out that the appropriate method for this purpose will depend entirely on the particular way in which the process of randomization is introduced.

We have seen how successfully these principles have been used in the case of field trials. It is essential that the same principles should also be applied in

the case of experiments of all other kinds. There is great scope for work in this direction in India. For, I am afraid, the need of statistical methods in experiments other than field trials has not yet been sufficiently recognized in this country. Much effort and time have been wasted in consequence.

Need of definite statistical objects

In fact it would be a salutary practice in most experimental studies to refrain from taking any measurements or recording any observations whatsoever until one was satisfied that these could be utilized in a valid manner for some useful purpose. In any case, it would be a safe rule to carry out a trial analysis with available material at the earliest opportunity. If this was done, it would often reveal gaps in the data or defects in the method of collecting them which could be often put right in time. If one waited until the end it would usually be too late.

Indeed in India it is tragic to see the enormous amount of statistical material which is collected at considerable expense but which is never used, or which can never be used in any way except as food for white ants. It would save a great deal of labour and money if no measurements or observations were recorded without a definite statistical purpose in view.

As already pointed out, in order to secure this end, the process of randomization and the projected method of analysis must be such that it would be possible to make precise statements as to the significance or non-significance of the results. When, as is usually the case, some previous information is already available, it is further necessary that the experiment should be designed in such a way that the expected precision is adequate for the purpose in view.

Statistics as a tool and not the end

Even this is not sufficient, something more is necessary. Before starting an experiment each worker should satisfy himself that, if the experiment is successful, something will be gained which is worth the time, labour, and money spent on it. I frankly confess that I have sometimes wondered whether this condition had been really fulfilled in the case of some of the agricultural experiments which I have had occasion to examine. I know that this is treading on dangerous grounds, but I do not think it can be emphasized too much that statistics is merely a means and not an end in itself. Wishart and Sanders [1936] have wisely remarked: 'In these days it is difficult, but very important to keep a sense of proportion over the question of experimentation. The statistical side has been given so much prominence in recent years that there is a real danger of statistics being regarded as the main interest in experimentation'.

Safeguards against statistical excesses

Agriculturists must not therefore allow statistics to degenerate into a kind of mysterious cult. The fundamental principles are easy to understand, and there is no reason why the experimenter should not take an intelligent interest in the designing of experiments. The statistician, owing chiefly to constant practice, is more skilled in handling certain technical tools which can be safely left to him. But it is the experimenter who is in a better position to judge the value of the experiment as a whole in its wider aspects.

Fortunately statistics itself may be used as a check against its own excess. It is possible, and possible only by statistical methods, to determine with

scientific precision the marginal (or additional) cost in money and human labour for obtaining any given amount of additional information or increased accuracy. In this way a kind of scientific cost accounting of experiments can be made possible, so that the experimenter may be guided in his decision by rational considerations.

Cooperation between agriculturists and statisticians

In India there is always a danger of our not being able to see the wood because of the trees. The only corrective is to keep the basic problem prominently in view. The experimenter should constantly remind the statistician (and also himself perhaps occasionally) that improving the standard of living of the 350 millions of our countrymen by increasing the produce of the earth is the ultimate aim of all agricultural experiments; and that how far progress is made in this direction is the final test by which all work will have to be judged. This is the only way in which the agriculturist will be able to breathe life into the dry bones of statistics. I therefore plead for a close, friendly, active, and fruitful cooperation between the agriculturist and the statistician in this task.

APPENDIX I

Mean squares in analysis of variance: Chinsurah complex experiment on rice, 1933-36

	Degrees of freedom	1933	1934	1936
Block	2	144098	359084	75533
Date of planting	4	2389142**	499687**	5417487**
Error	8	30461	40555	23907
Variety	2	708517**	564263**	385807**
Error	4	12455	19494	16339
Planting × Varieties	8	29608**	37276**	26112**
Error	16	4259	8181	3018
Spacing	2	64848**	4061	10809**
Seedling	2	32691**	5733	2028
Spacing × Seedling	4	1396	3221	905
Planting × Seedling	8	2484	5570	1236
Planting × Spacing	8	5320*	1814	970
Variety × Seedling	4	1112	10710*	1065
Variety × Spacing	4	1306	4898	1331
Planting × Variety × Seedling	16	3991*	2889	1305
Planting × Variety × Spacing	16	2952*	5347	1751
Planting × Seedling × Spacing	16	1442	1585	848
Variety × Seedling × Spacing	8	1198	4634	1050
Planting × Variety × Seedling × Spacing	32	947	2289	1779
Error	240	1364	2847	1882

* Indicates significance at 5 per cent level. ** Indicates significance at one per cent level.

NOTE.—The results are given for the three seasons 1933, 1934, and 1936 as the experiment failed in 1935 owing to drought. The test of significance indicates that the effects of the three varieties of rice, of the five dates of planting, and of their mutual interaction of the first order were appreciable in all the three seasons under observation. Variation of spacing also showed significant differences in 1933 and 1936 but not in 1934. Other effects were either insignificant or significant only for one season. A detailed examination showed that the variety called *bhasamanik* gave the highest yields at Chinsurah during all the three seasons. The first week of August was found to be the best date of planting; in fact the yield showed a distinct tendency to be lower if the transplanting was finished earlier or delayed by a fortnight. A close spacing and an increased number of seedlings per hole were necessary to insure against late transplantings, particularly in years of adverse rainfall distribution. But under a favourable monsoon, small variations in spacing or seedling numbers produced practically no differences in yield. Finally the superiority of one variety over another was not identical for all the dates of planting but was found to be significantly associated with the time of transplanting.

APPENDIX II

Note on variance, standard error, covariance, etc.

Let x_1, x_2, \dots, x_n be the yields of n plots. Then the average yield is defined as $x = \frac{x_1 + x_2 + \dots + x_n}{n}$ (1).

The 'deviation' (or 'error') of the yield is simply the difference between any individual yield and the average, i.e. $(x_1 - x), (x_2 - x)$, etc. The 'sum of squares of deviations' is given by $(x_1 - x)^2 + (x_2 - x)^2 + \dots + (x_n - x)^2$ (2).

The 'variance' of the yield is defined as the sum of squares of deviation divided by $(n-1)$, or

$$v = \frac{(x_1 - x)^2 + (x_2 - x)^2 + \dots + (x_n - x)^2}{(n-1)} \dots\dots\dots (3).$$

Here $(n-1)$ represents the 'degrees of freedom' which can be usually identified with the number of independent comparisons possible in any given case. In the present example, we can clearly have $(n-1)$ independent comparisons between the yields of n different plots.

It will be noticed that 'variance' represents a kind of average of the squares of deviations. The 'standard deviation' or 'standard error' is defined as the square root of the variance (which is sometimes also called the 'root-mean-square-deviation' or 'root-mean-square-error').

The variance defined in equation (3) is the variance of individual plots, and the corresponding standard deviation or standard error obtained by extracting the square root is the 'standard error per plot'.

The 'variance of the mean' of the yields, i.e. of x is obtained by dividing the variance of individual plots by n , the total number of plots concerned, i.e. is given by

$$\frac{v}{n} = \frac{(x_1 - x)^2 + (x_2 - x)^2 + \dots + (x_n - x)^2}{n(n-1)} \dots\dots\dots (4).$$

The 'standard error of the mean' is the square root of the above expression.

When more than one character (or variate) is present, the covariance of any two characters (or any two variates) is similarly defined as the sum of the products of the corresponding deviations divided by the degrees of freedom.

Thus if $(x_1, y_1) (x_2, y_2) \dots (x_n, y_n)$ are the n pairs of values of the two characters, and \bar{x} and \bar{y} their respective averages, then the covariance is given by

$$\frac{(x_1 - \bar{x})(y_1 - \bar{y}) + (x_2 - \bar{x})(y_2 - \bar{y}) + \dots + (x_n - \bar{x})(y_n - \bar{y})}{(n-1)} \dots (5).$$

Main effect and interaction.—If we have p treatments (or factors) then the main effect of each treatment (or factor) is the mean value of the relevant treatment (or factor) for all combinations of the other factors. The main effect is thus obtained by taking the average over all plots in which this particular factor occurs.

When two or more factors or treatments are used at the same time as in complex (factorial) experiments, the total effect due to the joint influence of two (or more) factors may or may not be equal to the sum of the effects due to each of the factors taken separately. Interaction between the factors is defined as the magnitude of the departure (if any) from the total effect calculated on the additive hypothesis. When the different factors act independently the joint effect will be necessarily additive, and the interaction will be consequently nil.

In an experiment involving p factors, we can consider the factors in pairs, and we shall have one first-order interaction corresponding to each pair or $\frac{p(p-1)}{2}$ first-order interactions altogether. We can also consider the factors by threes, and in this case, if the first-order interactions are affected by the presence of the third factor, then we shall get second-order interactions. In the same way we can also consider higher order interactions, but usually high order interactions are of little practical importance.

REFERENCES

- Allen, R. E. and Wishart, J. (1930). *J. Agric. Sci.* **20** (3), 130, 399
 Christidis, B. G. (1931). *J. Agric. Sci.* **21** (1), 14
 Fisher, R. A. (1923). *J. Agric. Sci.* **13**, 311
 ——— (1926). *J. Min. Agric.* **33**, 503
 ——— (1933). *Rothamsted Experimental Station Report*, p. 5
 ——— (1935). *The Design of Experiments*. Edinburgh: Oliver and Boyd.
 Report of the Royal Commission on Agriculture, 1928
 Vaidyanathan, M. (1934). *Report Imp. Coun. Agric. Res.*
 Wishart, J. and Sanders, H. G. (1936). *Principles and Practice of Field Experimentation*.
 Yates, F. (1933). *Emp. Expt. Agric.* **1**, (2) 129
 ——— (1935). *J. Roy. Stat. Soc., Supp. II* (2), 181
 ——— (1937). *Imp. Bur. Soil Sci. Tech. Comm.*, No. 35

A NOTE ON THE ANALYSIS OF 3^3 AND 3^4 DESIGNS
(WITH THREE-FACTOR INTERACTIONS
CONFOUNDED) IN FIELD EXPERIMENTS
IN AGRICULTURE

BY

M. VAIDYANATHAN, M.A., L.T., F.S.S.

AND

S. SUBRAMONIA IYER, M.A., F.S.S.

Imperial Council of Agricultural Research

(Received for publication on 19 December 1939)

INTRODUCTION

ON the recommendation of the Joint Committee of Field Experiments in Agriculture of the Imperial Council of Agricultural Research, certain suggestions for replanning of manurial experiments for rice and sugarcane were made to the provinces and states, and most of these have been accepted for adoption from 1939-1940 season. Of the designs suggested by the Committee, what are now known as 'factorial designs' are important. These are on the lines of the designs adopted at the Rothamsted Experimental Station in England, which the senior author of this paper had the opportunity of examining in detail at close quarters during 1937. As the idea of 'confounding' the effects with block differences is comparatively new to the agricultural experimenter in India, it may be stated even in the beginning that the object of 'confounding' with the blocks is to reduce the block-size which will otherwise become huge in a factorial design involving a number of levels of different factors. This is done without disturbing the interpretation of main effects and interactions on which we are interested. Thus with a 3^4 design, (i.e. with 3 levels of each of 4 factors), in the usual randomised block method there would be 81 plots in a block, which is obviously too huge for a correct interpretation; but if it is possible to confound some of the higher order interactions (say three or four factor interactions) with the blocks, without disturbing the main effects and lower order interactions on which we are interested, the block size can be reduced with only those treatments included in a block giving the desired comparisons. Similarly in a 3^3 design, even 27 plots are too huge for a block and we may confound with the blocks some of the three-factor interactions.

Again, where we are dealing with a 3^4 design, if it is not possible to replicate and find sufficient space for the experiment, Fisher has shown that even with a single set of 81 plots (without replications) it will be a valid interpretation of the data if higher-order interactions are treated as error [See 'Design of Experiments' p. 115 (second edition).]

But if it is possible to find space for a number of replications, then we may adopt what is known as 'partial confounding', by which interactions confounded in one block are not confounded in the rest, and all 'confounded interactions' are distributed over all the blocks, so that it may be possible to obtain some information for interactions confounded in one block from the blocks where they are not confounded.

The principles of planning and analysis of 3^3 and 3^4 designs have been dealt with by Yates in '*The Design and Analysis of Factorial Experiments*' (Technical Communication No. 35 of the Imperial Bureau of Soil Science). The object of this note is to explain fully the computations involved in the analysis of such designs so that they may be easily understood by the agricultural experimenter in India. Part I deals with 3^3 design and Part II with 3^4 design.

PART I

Analysis of 3^3 design (27 plots) split up into 3 sub-blocks of 9 plots each, such that 2 degrees of freedom of three-factor interactions are confounded with the three sub-blocks

We shall first illustrate the methods of analysis in the case of a 3^3 design (i.e. total of 27 plots) split up into 3 sub-blocks of 9 plots each such that 2 degrees of freedom of three-factor interactions are confounded with the three sub-blocks (note when 2 degrees of freedom are confounded with the blocks we want 3 sub-blocks, for 3 degrees of freedom 4 sub-blocks and so on).

MATERIAL

The data relate to an intercultural experiment on sugarcane conducted at Shahjahanpur, United Provinces, during 1938-39. The details of treatments are shown in Table I with the actual lay-out and plot yields.

The following are the details regarding the experiment :—

Size of plot 63 ft. \times 24½ ft. = 1/28.24 or 0.0354 acre

Size of plot harvested 59 ft. \times 17½ ft. = 1/42.19 or 0.0237 acre

Basal manuring—*Nil*.

Irrigation—*Vide* treatments in the experiment

Variety of cane—Co 312

System of planting.—Planted on flat 3½ ft. apart in rows on 27th January 1938

Experiment—Unreplicated into higher order interactions used as 'error'

The following statistical procedure is suggested in analysing the data :—

Formation of totals

Table I gives the plan of layout and plot yields; d, s, n standing for inter-culture, irrigation and nitrogen respectively. d, s, n are written in the same order and rank throughout. From Table I we obtain Table II, arranged in a conventional way; d_0, d_1, d_2 written down, s_0, s_1, s_2 across, and n_0, n_1, n_2 further up also across. This way of writing down the data of yield of treatments has the advantage that we can form from it two-way tables, i.e. with two factors only with facility. (1), (2) and (3) of Table III give two-way

totals of ds , dn and sn with their marginal totals. (4) and (5) of Table III gives for each of n_0 , n_1 , and n_2 diagonal totals I and J formed out of the nine values (d_0, d_1, d_2) (s_0, s_1, s_2) as explained below :—

If the nine values are written in the following order :—

$$\begin{array}{ccc}
 1 & 4 & 7 \\
 \text{(A) } 2 & 5 & 8 \\
 3 & 6 & 9
 \end{array}$$

then $I_1 = 1 + 5 + 9$ } $J_1 = 1 + 6 + 8$
 $(I) \quad I_2 = 2 + 6 + 7$ } $J_2 = 2 + 4 + 9$
 $I_3 = 3 + 4 + 8$ } $J_3 = 3 + 5 + 7$

It may be noted that (A), (I) and (J) have the first columns identical, second and third columns of both (I) and (J) are permutations of second and third columns of (A), I_1 is top left-bottom right diagonal of (A), J_3 is top-right bottom left diagonal and that row-wise both (I) and (J) add up to 15.

These I and J totals will be found useful for calculating two-factor interactions. Similarly for calculating three-factor interactions, we form diagonal totals out of (n_0, n_1, n_2) (I_1, I_2, I_3) , (n_0, n_1, n_2) , (J_1, J_2, J_3) in the same way as original I and J were formed. We thus obtain W_1, W_2, W_3 ; X_1, X_2, X_3 ; Y_1, Y_2, Y_3 and Z_1, Z_2, Z_3 as given in Table IV. Now the totals X_1, X_2, X_3 are those of the three sub-blocks, showing that the particular 2 degrees of freedom are confounded with the blocks.

Analysis of variance

First calculate the total sum of squares of the 27 values in the usual way [i.e. square each of the 27 values, add all the squares, and subtract the correction factor :—

$$\frac{(\text{total of the 27 values})^2}{27}$$

Then Table V gives sums of squares for the main effects and two-factor interactions calculated from the totals of the three two-way Tables of Table IIIa (See details of working).

The sum of squares corresponding to the confounded and unconfounded pairs of degrees of freedom of the three-factor interactions are found from Table IV, from (W), (X), (Y), (Z), remembering that W_1, W_2, \dots are totals of nine plots.

(Check :—The total sum of squares for 26 degrees of freedom on the basis of 26 values should tally with the sums of squares for 26 degrees of freedom got independently as explained above).

Table V further gives the analysis of variance into two-factor and three-factor interactions split up respectively into I, J ; W, X, Y, Z . Though it may not be necessary always to split up into above sets the process will help an understanding of the method of analysis, and explain the procedure of 'confounding'. Of the three-factor interactions, as 2 degrees of freedom are confounded with blocks, the other 6 degrees of freedom are taken to constitute 'error'. On this basis, in this particular case none of the effects is significant at $P = 0.05$.

TABLE I

Lay out	Field (G 6)			
$d_0s_0n_0$ 20.68	$d_2s_0n_1$ 20.20	$d_0s_1n_1$ 21.00	Sub-block I	
$d_1s_1n_0$ 20.45	$d_0s_2n_2$ 20.82	$d_2s_1n_2$ 18.88		
$d_1s_2n_1$ 19.77	$d_2s_2n_0$ 23.20	$d_1s_0n_2$ 19.30		
$d_0s_0n_2$ 19.25	$d_2s_0n_0$ 13.75	$d_1s_0n_1$ 18.87	Sub-block II	
$d_0s_2n_1$ 22.19	$d_1s_2n_0$ 21.67	$d_2s_2n_2$ 19.76		
$d_1s_1n_2$ 19.50	$d_2s_1n_1$ 19.78	$d_0s_1n_0$ 20.63		
$d_1s_1n_1$ 21.95	$d_1s_0n_0$ 18.45	$d_2s_0n_2$ 17.20	Sub-block III	
$d_2s_2n_1$ 22.70	$d_2s_1n_0$ 21.53	$d_1s_2n_2$ 24.52		
$d_0s_2n_0$ 20.75	$d_0s_1n_2$ 20.75	$d_0s_0n_1$ 17.97		

Treatments

Interculture	Irrigation	Nitrogen
Akola Hoe (d_0)	2 irrigations (s_0)	0 lb. (n_0)
Pt Junier (d_1)	4 irrigations (s_1)	100 lb. (n_1)
Kashi (d_2)	6 irrigations (s_2)	200 lb. (n_2)

TABLE II

Yields of separate treatment combinations

	n_0			n_1			n_2		
	s_0	s_1	s_2	s_0	s_1	s_2	s_0	s_1	s_2
d_0	20.68	20.63	20.75	17.97	21.00	22.19	19.25	20.75	20.82
d_1	18.45	20.45	21.67	18.87	21.95	19.77	19.30	19.50	24.52
d_2	13.75	21.53	23.20	20.20	19.78	22.70	17.20	18.88	19.76

TABLE III

Two-way tables for calculating main effects and two factor interactions

		s_0	s_1	s_2	
(1)	d_0	57.90	62.38	63.76	184.04
	d_1	56.62	61.90	65.96	184.48
	d_2	51.15	60.19	65.66	177.00
		n_0	n_1	n_2	
(2)	d_0	62.06	61.16	60.82	
	d_1	60.57	60.59	63.32	
	d_2	58.48	62.68	55.84	
(3)	s_0	52.88	57.04	55.75	165.67
	s_1	62.61	62.73	59.13	184.47
	s_2	65.62	64.66	65.10	195.38
		n_0	n_1	n_2	
(4)	I_1	64.33	62.62	58.51	
	I_2	60.73	60.84	59.00	
	I_3	56.05	60.97	62.47	
(5)	J_1	63.88	57.52	62.65	
	J_2	62.28	62.57	59.81	
	J_3	54.95	64.34	57.52	
		181.11	184.43	179.98	

TABLE IV

Three-factor interactions

	1	2	3
(W)	187.64	180.21	177.67
(X)	184.30	185.82	175.40
(Y)	183.97	189.27	172.28
(Z)	188.03	177.32	180.17

TABLE V

Analysis of variance

		Degrees of freedom	Sum of squares	Mean square	v_1/v_2
Main effects	$\left\{ \begin{array}{l} D \\ S \\ N \end{array} \right.$	2 2 2	3.9150 50.1908 1.1890	1.9575 25.0954 0.5945	Even this high ratio shows 'Not significant'.
Two factor interactions	$\left\{ \begin{array}{l} DS \\ DN \\ SN \end{array} \right.$	$\left\{ \begin{array}{l} I \\ J \end{array} \right.$	2 2	2.2489 4.2375	1.6216
		$\left\{ \begin{array}{l} I \\ J \end{array} \right.$	2 2	1.9389 6.7471	2.1715
		$\left\{ \begin{array}{l} I \\ J \end{array} \right.$	2 2	0.2969 4.4783	1.1938
DSN Three factor interactions	$\left\{ \begin{array}{l} W \text{ (Error)} \\ X \text{ (Confounded with blocks)} \\ Y \text{ (Error)} \\ Z \text{ (Error)} \end{array} \right.$	2 2 2 2	5.9651 7.0406 16.7925 6.8373	4.9325 (W, Y, Z)	
		26	111.8779		

Conclusions

None of the effects are significant even at $P = 0.05$

DETAILS OF WORKING

Table III (from Table II)

- (1) $d_0 s_0 = 20.68 + 17.97 + 19.25 = 57.90$
 $d_0 s_1 = 20.63 + 21.00 + 20.75 = 62.38$
 $d_0 s_2 = 20.75 + 22.19 + 20.82 = 63.76$
 and so on
- (2) $d_0 n_0 = 20.68 + 20.63 + 20.75 = 62.06$
 $d_0 n_1 = 17.97 + 21.00 + 22.19 = 61.16$
 $d_0 n_2 = 19.25 + 20.75 + 20.82 = 60.82$
 and so on
- (3) $s_0 n_0 = 20.68 + 18.45 + 13.75 = 52.88$
 $s_0 n_1 = 17.97 + 18.87 + 20.20 = 57.04$
 $s_0 n_2 = 19.25 + 10.30 + 17.20 = 55.75$
 and so on

(From Table II)

(4) & (5)

$$I_1 n_0 = 20.68 + 20.45 + 23.20 = 64.33$$

$$I_2 n_0 = 18.45 + 21.53 + 20.75 = 60.73$$

$$I_3 n_0 = 13.75 + 20.63 + 21.67 = 56.05$$

$$I_1 n_1 = 17.97 + 21.95 + 22.70 = 62.62$$

$$I_2 n_1 = 18.87 + 19.78 + 22.19 = 60.84$$

$$I_3 n_1 = 20.20 + 21.00 + 19.77 = 60.97$$

$$I_1 n_2 = 19.25 + 19.50 + 19.76 = 58.51$$

$$I_2 n_2 = 19.30 + 18.88 + 20.82 = 59.00$$

$$I_3 n_2 = 17.20 + 20.75 + 24.52 = 62.47$$

$$J_1 n_0 = 20.68 + 21.53 + 21.67 = 63.88$$

$$J_2 n_0 = 18.45 + 20.63 + 23.20 = 62.28$$

$$J_3 n_0 = 13.75 + 20.45 + 20.75 = 54.95$$

$$J_1 n_1 = 17.97 + 19.78 + 19.77 = 57.52$$

$$J_2 n_1 = 18.87 + 21.00 + 22.70 = 62.57$$

$$J_3 n_1 = 20.20 + 21.95 + 22.19 = 64.34$$

$$J_1 n_2 = 19.25 + 18.88 + 24.52 = 62.65$$

$$J_2 n_2 = 19.30 + 20.75 + 19.76 = 59.81$$

$$J_3 n_2 = 17.20 + 19.50 + 20.82 = 57.52$$

TABLE IV

From (4) of Table III

$$W_1 = 64.33 + 60.84 + 62.47 = 187.64$$

$$W_2 = 60.73 + 60.97 + 58.51 = 180.21$$

$$W_3 = 56.05 + 62.62 + 59.00 = 177.67$$

$$X_1 = 64.33 + 60.97 + 59.00 = 184.30$$

$$X_2 = 60.73 + 62.62 + 62.47 = 185.82$$

$$X_3 = 56.05 + 60.84 + 58.51 = 175.40$$

$$Y_1 = 63.88 + 62.57 + 57.52 = 183.97$$

$$Y_2 = 62.28 + 64.34 + 62.65 = 189.27$$

$$Y_3 = 54.95 + 57.52 + 59.81 = 172.28$$

$$Z_1 = 63.88 + 64.34 + 59.81 = 188.03$$

$$Z_2 = 62.28 + 57.52 + 57.52 = 177.32$$

$$Z_3 = 54.95 + 62.57 + 62.65 = 180.17$$

TABLE V

For D (2 D. F.)—

$$= \frac{(184.04)^2 + (184.48)^2 + (177.00)^2}{9} \text{ — C.F.}$$

$$= 11025.8435 \text{ — } 11021.9285$$

$$= 3.9150$$

For S (2 D. F.)—

$$= \frac{(165.67)^2 + (184.47)^2 + (195.38)^2}{9} \text{ — C.F.}$$

$$= 11072.1193 \text{ — } 11021.9285$$

$$= 50.1908$$

For N (2 D. F.)—

$$\frac{(181.11)^2 + (184.43)^2 + (179.98)^2}{9} \text{--- C.F.}$$

$$= 11023.1175 - 11021.9285$$

$$= 1.1890$$

For DS $\begin{cases} I \\ J \end{cases}$, we get from (1) of Table III

$$I_1 = 57.90 + 61.90 + 65.66 = 185.46$$

$$I_2 = 56.62 + 60.19 + 63.76 = 180.57$$

$$I_3 = 51.15 + 62.38 + 65.96 = 179.49$$

Then DS (I) (2 D. F.)—

$$\frac{(185.46)^2 + (180.57)^2 + (179.49)^2}{9} \text{--- C.F.}$$

$$= 11024.1774 - 11021.9285$$

$$= 2.2489$$

Similarly,

$$J_1 = 57.90 + 60.19 + 65.96 = 184.05$$

$$J_2 = 56.62 + 62.38 + 65.66 = 184.66$$

$$J_3 = 51.15 + 61.90 + 63.76 = 176.81$$

DS (J) (2 D. F.)

$$\frac{(184.05)^2 + (184.66)^2 + (176.81)^2}{9} \text{--- C.F.}$$

$$= 11026.1660 - 11021.9285$$

$$= 4.2375$$

For DN $\begin{cases} I \\ J \end{cases}$, we get from (2) of Table III

$$I_1 = 62.06 + 60.59 + 55.84 = 178.49$$

$$I_2 = 60.57 + 62.68 + 60.82 = 184.07$$

$$I_3 = 58.48 + 61.16 + 63.32 = 182.96$$

DN (I) (2 D. F.)

$$\frac{(178.49)^2 + (184.07)^2 + (182.96)^2}{9} \text{--- C.F.}$$

$$= 11023.8674 - 11021.9285$$

$$= 1.9389$$

Similarly,

$$J_1 = 62.06 + 62.68 + 63.32 = 188.06$$

$$J_2 = 60.57 + 61.16 + 55.84 = 177.57$$

$$J_3 = 58.48 + 60.59 + 60.82 = 179.89$$

DN (J) (2 D. F.)

$$\frac{(188.06)^2 + (177.57)^2 + (179.89)^2}{9} \text{--- C.F.}$$

$$= 11028.6756 - 11021.9285$$

$$= 6.7471$$

For $SN \begin{cases} I \\ J \end{cases}$, we get from (3) of Table III.

$$I_1 = 52.88 + 62.73 + 65.10 = 180.71$$

$$I_2 = 62.61 + 64.66 + 55.75 = 183.02$$

$$I_3 = 65.62 + 57.04 + 59.13 = 181.79$$

$SN (I) (2 \text{ D. F.})$

$$\begin{aligned} & \frac{(180.71)^2 + (183.02)^2 + (181.79)^2}{9} - \text{C.F.} \\ &= 11022.2254 - 11021.9285 \\ &= 0.2969 \end{aligned}$$

Similarly,

$$J_1 = 52.88 + 64.66 + 59.13 = 176.67$$

$$J_2 = 62.61 + 57.04 + 65.10 = 184.75$$

$$J_3 = 65.62 + 62.73 + 55.75 = 184.10$$

$SN (J) (2 \text{ D. F.})$

$$\begin{aligned} & \frac{(176.67)^2 + (184.75)^2 + (184.10)^2}{9} - \text{C.F.} \\ &= 11026.4068 - 11021.9285 \\ &= 4.4783 \text{ (From Table IV)} \end{aligned}$$

$D. S. N.$

$W (2 \text{ D. F.})$

$$\begin{aligned} & \frac{(187.64)^2 + (180.21)^2 + (177.67)^2}{9} - \text{C.F.} \\ &= 11027.8936 - 11021.9285 \\ &= 5.9651 \end{aligned}$$

$X (2 \text{ D. F.})$ (Confounded with blocks)

$$\begin{aligned} & \frac{(184.30)^2 + (185.82)^2 + (175.40)^2}{9} - \text{C.F.} \\ &= 11028.9651 - 11021.9285 \\ &= 7.0406 \end{aligned}$$

$Y (2 \text{ D. F.})$

$$\begin{aligned} & \frac{(183.97)^2 + (189.27)^2 + (172.28)^2}{9} - \text{C.F.} \\ &= 11038.7213 - 11021.9285 = 16.7928 \end{aligned}$$

$Z (2 \text{ D. F.})$

$$\begin{aligned} & \frac{(188.03)^2 + (177.32)^2 + (180.17)^2}{9} - \text{C.F.} \\ &= 11028.7658 - 11021.9285 \\ &= 6.8373 \end{aligned}$$

PART II

Analysis of 3^4 design (81 plots in one complete replication : Two such replications)

As no experimental data are still available in India to illustrate the analysis of a 'confounded 3^4 design,' a set of uniformity trial data on sugarcane has been taken to explain the procedure. (For the data please see *Ind. J. Agric. Sci.* 6 part 3, 1936).

In this case, we have taken two complete replications. The degrees of freedom confounded are :—

8 D. F.

$\left. \begin{array}{l} ABC \ (W) \\ ABD \ (Y) \\ ACD \ (Z) \\ BCD \ (X) \end{array} \right\}$	Confounded in the first replication.
---	--------------------------------------

8 D. F.

$\left. \begin{array}{l} ABC \ (X) \\ ABD \ (Z) \\ ACD \ (W) \\ BCD \ (Y) \end{array} \right\}$	Confounded in the second replication.
---	---------------------------------------

(W, X, Y, Z, have been explained in the first part).

It will be noted that there are nine sub-blocks of nine plots each in each complete replication thus confounding the 8 D. F.

Total 'sum of squares'

(1) Table Ia gives the yields of each plot (162 plots). (For convenience of numerical work deviations from 300.0 have been used).

The sum of squares due to 161 degrees of freedom is calculated from the 162 plot yields in the usual way, (i.e. the actual sum of squares of all the plot yields minus T^2/n where T is the grand total and n the number of plots). Similarly the sum of squares due to the 18 blocks can be calculated.

Sum of squares due to 81 treatments

(2) Table IIa gives the yields of the 81 treatment combinations ($3 \times 3 \times 3 \times 3$), tabulated in the standard order as in the case of a 3^3 design. Sum of squares due to 80 degrees of freedom may be calculated from this table, and in the present case as some interactions are confounded with the blocks, and hence it will be useful only for checking, not necessary for the final analysis of variance. The correction factor is the same as in (1) above, viz. (grand total)²/162). Each figure in this table being the total of two plot yields (two being the number of replications), the sum of the squares of these 81 totals should be divided by two before applying the correction factor.

Main effects and two-factor interactions

(3) Table IIIa gives the six two-way tables possible for all the six pairs of the four factors. Each figure in these tables is the total of 18 plot yields, the marginal totals being the sum of 54 plot yields. The sum of squares due to main effects A, B, C and D are calculated from the appropriate marginal totals (the divisor being 54, with the usual correction factor).

Sum of squares due to six two-factor interactions AB, BC, \dots , etc., are calculated by forming the diagonal totals I 's and J 's as shown in the table.

Three-factor interactions

(4) Table IVa gives the four three-factor tables for calculating the three-factor interactions ABC , ABD , ACD and BCD .

As in the case of two-way tables the grand totals of each of these tables is the same as the grand total of all the 162 plots; each figure representing the total of six plots.

From each of the three-factor tables, the corresponding W , X , Y and Z (2 D. F. each) totals are calculated as explained in Part I, and the sum of squares ignoring confounding may be calculated from these in the usual way, and can be used for checking the calculations as to whether the separate sums of squares all add up to the 80 degrees of freedom for treatments as shown above.

Block totals

Table Va: This table is intended to obtain the three-factor interactions after the elimination of effects, confounded with the blocks. The nine block totals of each of the replications are numbered as shown in the plan and then written down in the standard order, viz. :—

1	4	7
2	5	8
3	6	9

The marginal totals and also I and J totals of the above arrangement are then calculated for each complete replication.

Table VIa shows the procedure adopted to correct the W , X , Y and Z totals of the three-factor interactions, in order to get W' , X' , Y' , Z' from which the unconfounded part of the sums of squares are calculated, e.g., for ABC ; W of ABC (2 D. F.) is confounded in block I. Hence subtract the J totals of block I (a) (Table Va) from W to get W' . The general rule is that the confounded parts are given by :—

BCD —Column totals (Table Va).

ACD —Row totals ,,

ABD — I totals ,,

ABC — J totals ,,

(See Table VI a).

The sum of squares due to the unconfounded portions of the three-factor interactions are to be calculated from these totals remembering that the number of plots is different according as to whether the particular W , X , Y or Z is the full value or after allowing for confounding.

These processes are exactly similar to the case of the 3^3 design, the operations being merely repeated for the four three-factor interactions given in Table IV-a.

Calculation of the sum of squares due to 16 degrees of freedom for the four-factor interactions

(8) Table VII-a: From Table II-a and I 's and J 's of (a b) corresponding to C 's and D 's taken in succession are entered in Table VII-a. From these W , X , Y , Z of (abc) corresponding to d_0 , d_1 , d_2 are then calculated. These may be designated as W_d , X_d , Y_d and Z_d for d_0 , d_1 and d_2 .

Table VIII-*a* gives a rearrangement of these values.

(9) Table IX-*a*.—The *I* and *J* totals of Table VIII-*a* for W_d , X_d , Y_d , and Z_d give the totals for calculating the sum of squares (8 pairs of 2 D. F. each) due to 16 degrees of freedom of the *ABCD* interaction.

Full analysis of variance table is given in Table X-*a*.

SUMMARY

1. The data of a 3^3 design used for a (cultural experiment on sugarcane at Shahjahanpur, during 1938-39) where some of the three-factor interactions are confounded in a single replication are used to illustrate the analysis in such cases.

2. Similarly for illustrating the procedure for analysing a confounded 3^4 design, as no data are so far available in India a set of uniformity trial data has been taken. The necessary statistical calculations have been given, and particular attention has been devoted to the computation of confounded and partially confounded three-factor interactions and unconfounded four-factor interactions.

3. The principles and notation employed in splitting the highest order interaction in a 3^n design have been explained in a logical way, in the Appendix.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. A. K. Mitra, Economic Botanist, Shahjahanpur for his kind permission to include his data in this paper.

REFERENCES

Yates (1937). *Design and Analysis of Factorial Experiments*, Technical Communication No. 35 of the Imperial Bureau of Soil Science.

APPENDIX

Explanation of notations I, J, W, X, Y, Z, etc.

If *I* and *J* denote the symbolic operators for forming the three diagonal totals in the standard way, from a 3×3 table written in the standard form, it is useful to see how the repeated operation of these symbols enables us to split the degrees of freedom of the highest order interaction in the case of a 3^n design.

Let (*ab*) denote the 3×3 table of 'a' and 'b'.

	b_0	b_1	b_2
a_0	1	4	7
a_1	2	5	8
a_2	3	6	9

Then *AB* (*I*) or in the notation we shall adopt *I* (*ab*) will stand for the diagonal totals I_1 , I_2 , I_3 of (*ab*):—

$$\begin{aligned} I_1 &= 1 + 5 + 9 \\ I_2 &= 2 + 6 + 7 \\ I_3 &= 3 + 4 + 8 \end{aligned}$$

Similarly $J(ab)$ stands for :—

$$J_1 = 1 + 6 + 8$$

$$J_2 = 2 + 4 + 9$$

$$J_3 = 3 + 5 + 7$$

(i) 3×3 design : Four degrees of freedom of the two-factor interactions are given by :—

$$I(ab) = 2 \text{ D. F.}$$

$$J(ab) = 2 \text{ D. F.}$$

(ii) $3 \times 3 \times 3$ design : Eight degrees of freedom of the three-factor interactions are given by :—

$$I \{ I(ab), c \} = W(abc)^* = 2 \text{ D. F.}$$

$$J \{ I(ab), c \} = X(abc) = 2 \text{ D. F.}$$

$$I \{ J(ab), c \} = Y(abc) = 2 \text{ D. F.}$$

$$J \{ J(ab), c \} = Z(abc) = 2 \text{ D. F.}$$

(iii) $3 \times 3 \times 3 \times 3$ design : Sixteen degrees of freedom of the four-factor interactions are given by :—

$$I \{ W(abc), d \} = O(abcd) = 2 \text{ D. F.}$$

$$J \{ W(abc), d \} = P(abcd) = 2 \text{ D. F.}$$

$$I \{ X(abc), d \} = Q(abcd) = 2 \text{ D. F.}$$

$$J \{ X(abc), d \} = R(abcd) = 2 \text{ D. F.}$$

$$I \{ Y(abc), d \} = S(abcd) = 2 \text{ D. F.}$$

$$J \{ Y(abc), d \} = T(abcd) = 2 \text{ D. F.}$$

$$I \{ Z(abc), d \} = U(abcd) = 2 \text{ D. F.}$$

$$J \{ Z(abc), d \} = V(abcd) = 2 \text{ D. F.}$$

* $ABC(W)$ in Yates' notation.

TABLE Ia

Data of plot yields (deviations from 300.0 lb.)

(First number indicates the treatment and the second the plot yield)

I replication				II replication				Third replication			
1	2	3	4	1	2	3	4	1	2	3	4
59 2 -39 15 16 84 30 38	82 60 28 10 41 71 54 78 63	9 1 5 2 6 7 8 9 3	60 116 6 20 43 45 68 102 40	7 20 -60 68 22 -20 3 146 56	-36 -7 -5 -12 -36 -57 -22 14 76	1 2 3 4 5 6 7 8 9	21 -7 -4 4 32 51 64 97 80	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9
4	5	6	7	8	9	10	11	12	13	14	15
12 47 28 108 52 103 46 94 20 28	46 35 108 14 34 64 87 38	4 1 2 3 4 5 6 7 8 9	46 89 25 75 51 77 63 103 88	44 22 72 120 106 38 26 43 5	35 57 73 92 28 26 39 21 11	1 2 3 4 5 6 7 8 9	39 54 24 25 192 47 53 -1 61	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9
7	8	9	10	11	12	13	14	15	16	17	18
36 27 58 57 38 13 -10	68 35 23 53 30 67 40 112	7 1 2 3 4 5 6 7 8 9	2 65 44 25 44 44 40 38 58	100 32 31 49 20 0 24 31 6	25 -30 -38 18 11 -10 -12	1 2 3 4 5 6 7 8 9	55 -31 61 50 5 -17 59 67	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8 9

First figure of treatment

Second figure of treatment

Thus treatment '25' means $a_1 b_2 c_1 d_1$.

TABLE II-a
Yields of 81 treatment combinations

	d_0								
	c_0			c_1			c_2		
	b_0	b_1	b_2	b_0	b_1	b_2	b_0	b_1	b_2
a_0	96	172	37	37	35	85	101	97	107
a_1	109	63	97	57	61	233	17	22	51
a_2	33	90	124	87	61	108	56	51	45
	d_1								
	c_0			c_1			c_2		
	b_0	b_1	b_2	b_0	b_1	b_2	b_0	b_1	b_2
a_0	71	13	107	103	89	23	80	86	97
a_1	67	52	121	—5	131	22	119	48	170
a_2	84	89	99	—54	110	64	96	82	114
	d_2								
	c_0			c_1			c_2		
	b_0	b_1	b_2	b_0	b_1	b_2	b_0	b_1	b_2
a_0	107	60	68	112	126	110	24	167	57
a_1	84	30	34	58	76	101	89	140	37
a_2	116	45	144	45	10	74	—43	113	57

TABLE III-a

	b_0	b_1	b_2
a_0	731	845	691
a_1	595	623	866
a_2	420	651	829
c_0	767	614	831
c_1	440	699	820
c_2	539	806	735

	b_0	b_1	b_2
d_0	593	652	887
d_1	561	700	817
d_2	592	767	682

	d_0	d_1	d_2
a_0	767	669	831
a_1	710	725	649
a_2	655	684	561

	c_0	c_1	c_2
c_0	821	703	688
c_1	764	483	712
c_2	547	892	641

	c_0	c_1	c_2
a_0	731	720	816
a_1	657	734	693
a_2	824	505	571

Main effects

A	(2 D. F)	= 1247.12
B	(2 D. F)	= 3827.27
C	(2 D. F)	= 593.05 *
D	(2 D. F)	= 77.57

Two-factor Interactions

AB	
I_1	= 731 + 623 + 829 = 2183
I_2	= 595 + 651 + 691 = 1937
I_3	= 420 + 845 + 866 = 2131
J_1	= 731 + 651 + 866 = 2248
J_2	= 595 + 845 + 829 = 2269
J_3	= 420 + 623 + 691 = 1734

For AB (4 D.F)(S.S. = S.S. due to I 's + S.S. due to J 's)

$$= \frac{26267240}{54} - 2C.F.$$

$$= 4022.95$$

BC

$$\begin{aligned}
 I_1 &= 767 + 699 + 735 = 2201 \\
 I_2 &= 440 + 806 + 831 = 2077 \\
 I_3 &= 539 + 614 + 820 = 1973 \\
 J_1 &= 767 + 806 + 820 = 2393 \\
 J_2 &= 440 + 614 + 735 = 1789 \\
 J_3 &= 539 + 699 + 831 = 2069
 \end{aligned}$$

For *BC* (4 D.F.) as before

$$\begin{aligned}
 &= \frac{26258790}{54} - 2 \text{ C. F.} \\
 &= 3866.47
 \end{aligned}$$

BD

$$\begin{aligned}
 I_1 &= 593 + 700 + 682 = 1975 \\
 I_2 &= 561 + 767 + 887 = 2215 \\
 I_3 &= 592 + 652 + 817 = 2061 \\
 J_1 &= 593 + 767 + 817 = 2177 \\
 J_2 &= 561 + 652 + 682 = 1895 \\
 J_3 &= 592 + 700 + 887 = 2179
 \end{aligned}$$

For *BD* (4 D.F.)

$$\begin{aligned}
 &= \frac{26132966}{54} - 2 \text{ C. F.} \\
 &= 1536.39
 \end{aligned}$$

AD

$$\begin{aligned}
 I_1 &= 767 + 725 + 561 = 2053 \\
 I_2 &= 710 + 684 + 831 = 2225 \\
 I_3 &= 655 + 669 + 649 = 1973 \\
 J_1 &= 767 + 684 + 649 = 2100 \\
 J_2 &= 710 + 669 + 561 = 1940 \\
 J_3 &= 655 + 725 + 831 = 2211
 \end{aligned}$$

for *AD* (4 D. F.) as before

$$\begin{aligned}
 &= \frac{26120284}{54} - 2 \text{ C. F.} \\
 &= 1301.54
 \end{aligned}$$

CD

$$\begin{aligned}
 I_1 &= 821 + 483 + 641 = 1945 \\
 I_2 &= 764 + 892 + 688 = 2344 \\
 I_3 &= 547 + 703 + 712 = 1962 \\
 J_1 &= 821 + 892 + 712 = 2425 \\
 J_2 &= 764 + 703 + 641 = 2108 \\
 J_3 &= 547 + 483 + 688 = 1718
 \end{aligned}$$

For *CD* (4 D.F.) as before

$$\begin{aligned}
 &= \frac{26402618}{54} - 2 \text{ C. F.} \\
 &= 6529.95
 \end{aligned}$$

AC

$$\begin{aligned}
 I_1 &= 731 + 734 + 571 = 2036 \\
 I_2 &= 657 + 505 + 816 = 1978 \\
 I_3 &= 824 + 720 + 693 = 2237 \\
 J_1 &= 731 + 505 + 693 = 1929 \\
 J_2 &= 657 + 720 + 571 = 1948 \\
 J_3 &= 824 + 734 + 816 = 2374
 \end{aligned}$$

For *AC* (4 D. F.) as before

$$\begin{aligned}
 &= \frac{26213570}{54} - 2 \text{ C. F.} \\
 &= 3029.06
 \end{aligned}$$

TABLE IV-a

	c_0			c_1			c_2		
	b_0	b_1	b_2	b_0	b_1	b_2	b_0	b_1	b_2
a_0	274	245	212	252	250	218	205	350	261
a_1	260	145	252	110	268	356	225	210	258
a_2	233	224	367	78	181	246	109	246	216

	d_0			d_1			d_2		
	b_0	b_1	b_2	b_0	b_1	b_2	b_0	b_1	b_2
a_0	234	304	229	254	188	227	243	353	235
a_1	183	146	381	181	231	313	231	246	172
a_2	176	202	277	126	281	277	118	168	275

	d_0			d_1			d_2		
	c_0	c_1	c_2	c_0	c_1	c_2	c_0	c_1	c_2
a_0	305	157	305	191	215	263	235	348	248
a_1	269	351	90	240	148	337	148	235	266
a_2	247	256	152	272	120	292	305	129	127

	d_0			d_1			d_2		
	c_0	c_1	c_2	c_0	c_1	c_2	c_0	c_1	c_2
b_0	238	181	174	222	44	295	307	215	70
b_1	325	157	170	154	330	216	135	212	420
b_2	258	426	203	327	109	381	246	285	151

TABLE IVa—*contd.**Three-factor interactions**ABC*

	c_0	c_1	c_2
I_1	786	766	631
I_2	696	509	732
I_3	730	684	717
J_1	750	789	709
J_2	872	606	791
J_3	590	564	580
W	2012	2011	2228
X	2202	2179	1870
Y	1936	2145	2170
Z	2105	2241	1905

ABD

	d_0	d_1	d_2
I_1	657	762	764
I_2	614	689	634
I_3	861	627	643
J_1	817	848	583
J_2	764	646	859
J_3	551	584	599
W	1989	2005	2257
X	1918	2019	2314
Y	2062	1931	2258
Z	2260	2211	1780

BCD

	d_0	d_1	d_2
I_1	598	933	670
I_2	925	558	490
I_3	609	587	881
J_1	834	547	1012
J_2	709	579	501
J_3	589	952	528
W	2037	2182	2032
X	1675	2739	1837
Y	1941	2673	1637
Z	2287	1784	2180

ACD

	d_0	d_1	d_2
I_1	808	631	597
I_2	830	623	525
I_3	494	824	919
J_1	651	648	630
J_2	578	747	623
J_3	903	683	788
W	2350	2251	1650
X	2157	2380	1714
Y	2186	1891	2174
Z	1957	2014	2280

TABLE V-a

Block I

312	425	334	1071
496	425	434	1355
500	617	395	1512

1308 1467 1163

$$I_1 = 312 + 425 + 395 = 1132$$

$$I_2 = 496 + 617 + 334 = 1447$$

$$I_3 = 500 + 425 + 434 = 1359$$

$$J_1 = 312 + 617 + 434 = 1363$$

$$J_2 = 496 + 425 + 395 = 1316$$

$$J_3 = 500 + 425 + 334 = 1259$$

Block II

242	471	293	1006
-30	382	-41	311
348	394	254	996

560 1247 506

$$I_1 = 242 + 382 + 254 = 878$$

$$I_2 = -30 + 394 + 293 = 657$$

$$I_3 = 348 + 471 - 41 = 778$$

$$J_1 = 242 + 394 - 41 = 595$$

$$J_2 = -30 + 471 + 254 = 695$$

$$J_3 = 348 + 382 + 293 = 1023$$

TABLE VI-a

ABC

<i>W</i>	2012	2011	2228
<i>(W)'</i>	1363	1316	1259 <i>J</i> 's totals of Table Va Block—I
	649	695	969
<i>X</i>	2202	2179	1870
<i>(X)'</i>	595	695	1023 <i>J</i> 's totals of Table Va Block—II
	1607	1484	847
<i>(Y)</i>	1936	2145	2170
<i>(Z)</i>	2105	2241	1905
<i>(W)</i>	$= 68266.18 - 66049 = 2217.18$		
<i>(X)</i>	$= 203782 - 191454.86 = 12327.10$		
<i>(Y)</i>	$= 241815.20 - 241203.71 = 611.49$		
<i>(Z)</i>	$= 242261.68 - 241203.71 = 1057.97$		

450

(F)	2003	2001	2000
T	1995	1997	1999 <i>Remains of Table V B1—I</i>
Z	1996	1998	1999
E	1996	1997	1998 <i>1/2 totals of Table V B1—II</i>
	1998	1999	2000
W	1996	1998	1999
H	1996	1999	2000
T	2000-01 — 2000-02 = 2000-02		
C	2000-02 — 2000-03 = 2000-03		
W	2000-03 — 2000-04 = 2000-04		
H	2000-04 — 2000-05 = 2000-05		

500

H	1995	1996	1997
E	1996	1997	1998 <i>Remains of Table V, Block I</i>
H	1997	1998	1999
T	1997	1998	1999 <i>Remains of Table V, Block II</i>
	1998	1999	2000
W	1997	1998	1999
Z	1997	1998	1999
T	2000-01 — 2000-02 = 2000-02		
C	2000-02 — 2000-03 = 2000-03		
W	2000-03 — 2000-04 = 2000-04		
H	2000-04 — 2000-05 = 2000-05		

450

E	1997	1998	1999
H	1997	1998	1999 <i>Remains of Table V, Block I</i>
	1998	1999	2000
W	1997	1998	1999
H	1997	1998	1999 <i>Block II</i>
	1998	1999	2000
T	1997	1998	1999
(F)	1998	1999	2000
E	2000-01 — 2000-02 = 2000-02		
W	2000-02 — 2000-03 = 2000-03		
H	2000-03 — 2000-04 = 2000-04		
(F)	2000-04 — 2000-05 = 2000-05		

TABLE VII-4

	δ_1		
	δ_1	δ_2	δ_3
C	264	260	266
E	258	255	256
H	262	256	266
J	263	261	266
J	258	255	256
J	264	259	265
W	260	259	262
X	253	249	253
(F)	268	241	252
(F)	255	261	259

	d_1		
	c_0	c_1	c_2
I_1	222	298	242
I_2	263	128	298
I_3	218	57	352
J_1	281	235	332
J_2	179	148	319
J_3	243	100	241
(W)	702	562	814
(X)	577	913	588
(Y)	670	611	797
(Z)	700	655	723
	d_2		
	c_0	c_1	c_2
I_1	281	262	221
I_2	197	178	259
I_3	210	272	161
J_1	186	223	174
J_2	288	258	313
J_3	214	231	154
(W)	620	690	731
(X)	812	620	609
(Y)	598	693	750
(Z)	730	665	646

TABLE VIII-a
Four-factor interactions

	d_0	d_1	d_2
W_1	690	702	620
W_2	759	562	690
W_3	683	814	731
X_1	813	577	812
X_2	646	913	620
X_3	673	588	609
Y_1	668	670	598
Y_2	841	611	693
Y_3	623	797	750
Z_1	675	700	730
Z_2	921	655	665
Z_3	536	723	646

TABLE IX-a

From W_d

$$\begin{aligned}
 I_1 &= 690 + 562 + 731 = 1983 \\
 I_2 &= 759 + 814 + 620 = 2193 \\
 I_3 &= 683 + 702 + 690 = 2075 \\
 J_1 &= 690 + 814 + 690 = 2194 \\
 J_2 &= 759 + 702 + 731 = 2192 \\
 J_3 &= 683 + 562 + 620 = 1865 \\
 484146.07 - 482407.42 &= 1738.65 \text{ (4 D. F.)}
 \end{aligned}$$

From X_d

$$\begin{aligned}
 I_1 &= 813 + 913 + 609 = 2335 \\
 I_2 &= 646 + 588 + 812 = 2046 \\
 I_3 &= 673 + 577 + 620 = 1870 \\
 J_1 &= 813 + 588 + 620 = 2021 \\
 J_2 &= 646 + 577 + 609 = 1832 \\
 J_3 &= 673 + 913 + 812 = 2398 \\
 487524.26 - 482407.42 &= 5118.84
 \end{aligned}$$

From Y_d

$$\begin{aligned}
 I_1 &= 668 + 611 + 750 = 2029 \\
 I_2 &= 841 + 797 + 598 = 2236 \\
 I_3 &= 623 + 670 + 693 = 1986 \\
 J_1 &= 668 + 797 + 693 = 2158 \\
 J_2 &= 841 + 670 + 750 = 2261 \\
 J_3 &= 623 + 611 + 598 = 1832 \\
 484926.70 - 482407.42 &= 2519.28
 \end{aligned}$$

From Z_d

$$\begin{aligned}
 I_1 &= 675 + 655 + 646 = 1976 \\
 I_2 &= 921 + 723 + 730 = 2374 \\
 I_3 &= 536 + 700 + 665 = 1901 \\
 J_1 &= 675 + 723 + 665 = 2063 \\
 J_2 &= 921 + 700 + 646 = 2267 \\
 J_3 &= 536 + 655 + 730 = 1921 \\
 485921.33 - 482407.42 &= 3513.91
 \end{aligned}$$

Total for 16 D. F. = 3513.91 + 2519.28 + 5118.84 + 1738.65
= 12890.68

TABLE X-a
Analysis of variance

Due to		Degrees of freedom	Sums of squares	Mean square
Total		161	213707.29	
Main effects	A	2	1247.12	
	B	2	3827.27	
	C	2	593.05	
	D	2	77.57	
Two-factor interactions	AB	4	4022.95	
	AC	4	3029.06	
	AD	4	1301.54	
	BC	4	3866.47	
	BD	4	1536.39	
	CD	4	6529.95	
Three-factor interactions	ABC (W')	2	2217.18	} Partially confounded.
	ABC (X')	2	12327.11	
	ABC (Y)	2	611.49	} Unconfounded.
	ABC (Z)	2	1057.97	
	ABD (W)	2	836.94	} Unconfounded.
	ABD (X)	2	1568.16	
	ABD (Y')	2	4593.85	} Partially confounded.
	ABD (Z')	2	5909.73	
	ACD (W')	2	30680.40	} Partially confounded.
	ACD (Z')	2	954.74	
	ACD (X)	2	4256.38	} Unconfounded.
	ACD (Y)	2	1032.46	
	BCD (X')	2	15689.85	} Partially confounded.
	BCD (Y')	2	1870.99	
	BCD (W)	2	268.83	} Unconfounded.
	BCD (Z)	2	1600.46	
Four-factor interactions	ABC(W) D	4	1738.65	
	ABC (X) D.	4	5118.84	
	ABC (Y) D.	4	2519.28	
	ABC (Z) D	4	3513.91	
	Blocks	17	52890.84	
Remainder		64	36417.86	
Total		161	213707.29	

W', X', etc., have been computed from the block where they are not confounded (for method of calculation see Table VI).

NOTES

NOTICE No. 4 OF 1939

THE following plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

I. LIST OF U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, SERVICE AND REGULATORY ANNOUNCEMENTS

1. *Quarantine and other official announcements*

- (i) *Japanese Beetle Quarantine (No. 48).*—Modification of regulations.
- (ii) *Pink Bollworm Quarantine (No. 52).*—Modifications of regulations:—
 - (a) to add okra to the list of articles interstate movement of which is restricted from regulated areas.
 - (b) to further extend the regulated areas.

2. *Summaries of plant quarantine import restrictions*

- (i) *British Colony of Bermuda.*—Revision of the digest.
- (ii) *Republic of Colombia.*—Authorised ports of entry.
- (iii) *Republic of Argentina.*—Plants and parts of plants of Rosaceae.
- (iv) *Colony and Protectorate of Kenya.*—Importation of soil prohibited—Fruits to be certified—Addition to restricted areas.
- (v) *Mexico.*—Extension Quarantine No. 12 amended—Alfalfa Weevil.
- (vi) *Cuba.*—Importation of cotton seed into Isle of Pines prohibited.

II. OTHER ANNOUNCEMENTS

Jamaica (B. W. I.).—Government Notice No. 116—Restriction of importation of seed potatoes.

Kenya.—Government Notice No. 468—Addition to the list of restricted seeds—Potatoes.

Malta.—Government Notice No. 461—Colorado Beetle—Import restrictions regarding plants etc.

THE 28th INDIAN SCIENCE CONGRESS, BENARES JANUARY, 1941

DISCUSSIONS IN THE AGRICULTURAL SECTION

THE Agricultural Sectional Committee proposes to hold Discussions on the following subjects during the next Session of the Indian Science Congress to be held at Benares early in January 1941. Scientific workers in India who desire to contribute papers to the above discussions are requested to communicate with the undersigned. The Rules of the Indian Science Congress Association require that authors of such contributions should be members of the Association of some category.

DISCUSSIONS

1. Drought resistance in plants.
2. The need for the exploration of wild forms for the improvement of crops.
3. Quality in crops.

10 February 1940
Indian Institute of Science,
Hebbal P. O., Bangalore.

C. N. ACHARYA,
Recorder,
Agricultural Section.

THE MAYNARD GANGA RAM PRIZE

APPLICATIONS are invited for the award of the Maynard Ganga Ram Prize of Rs. 2,000 for the two years ending 31st December 1940, for a discovery or an invention or a new practical method which will tend to increase agricultural production in the Punjab on a paying basis. Competition for the prize is restricted to non-officials only, irrespective of caste, creed or nationality. Government servants are not eligible on this occasion. Essays and theses are not accepted. The prize will be awarded for something practically achieved as a result of work done after the prize was founded in 1925. Competitors in their applications must give a clear account of the history of their invention or discovery and must produce clear evidence that it is the result of their own work. In the case of an improved crop details of parentage, evolution and history and a botanical description are necessary.

The Managing Committee reserves to itself the right of withholding or postponing the prize if no satisfactory achievement is reported to it.

Entries should reach the Director of Agriculture, Punjab, Lahore, not later than 31st December 1940.

WOODHOUSE MEMORIAL PRIZE

IN memory of Mr. E. J. Woodhouse, late Economic Botanist and Principal of Sabour Agricultural College, who was killed in action in France in 1917, a biennial prize in the form of a silver medal and books of a combined value of Rs. 100 will be awarded to the writer of the best essay on a subject to be selected from the list noted below. The length of the essay should not exceed 4,000 words.

The competition is open to graduates of Indian Universities and to Diploma holders and Licentiates of recognized Agricultural Colleges in India who are not more than 30 years of age on the date of submission of their essays.

Papers should be forwarded to the Director of Agriculture, Bihar, Patna, before the 30th June 1940.

Failing papers of sufficient merit, no award will be made. Essays must be typewritten on one side of paper only.

SUBJECTS FOR ESSAY

1. The importance of physiological studies in modern plant breeding.
2. Dominant species as an index of soil texture.
3. Modern methods of inducing mutations and polyploidy and their value for Indian agriculture.
4. Problems of wheat improvement in India.

REVIEWS

Biological Abstract

MEN engaged in research in medicine, public health, ecology, agriculture, forestry, botany or zoology, geography, and other fields, will welcome the announcement that *Biological Abstracts* is undertaking a more complete abstracting and segregation of the current research literature in bioclimatology and biometeorology. The section *Bioclimatology-Biometeorology* will appear within the section *Ecology* in *Biological Abstracts*, and will be under the editorship of Mr. Robert G. Stone of the Blue Hill Observatory, Harvard University.

The increasing interest in climatic and meteorological factors in their relation to biology, medicine, and agriculture is one of the significant trends of modern science. Ecologists have long appreciated the importance of temperature, humidity, radiation, barometric pressure, wind movement, and meteorological factors generally, as important factors in controlling the distribution and abundance of animals and plants. Foresters, horticulturists, and entomologists have likewise been concerned with the interrelationships of climatic and meteorological factors to the organisms with which they work. The developments of air conditioning and aviation have lately brought other important research groups into the field resulting in an increasing amount of research. This is often the work of individuals and groups not now in effective contact with biologists, and frequently appears in periodicals not commonly consulted by biologists.

In all civilized nations diverse research groups have sprung into being which, though they often devote much attention to the same fundamental natural forces, still work in practical isolation from each other, with a different background of training, and associations, belonging to different societies meeting at different times and places, publishing in different journals, reading different literature, investigating different types of things. These groups, however, are beginning to apply common ideas and common methods to the study of situations that are basically similar. For example, techniques and concepts derived from a study of the influence of weather factors on the spread of influenza or the common cold are likely to have a very high transfer value as applied to the study of the spread or survival of plant disease or economic insects. Conversely, it should be possible for research workers in the field of public health to make use of many findings of the entomologists, foresters, ecologists, plant pathologists, and other biological groups.

The abstracting journals of broad scope, like *Biological Abstracts*, are admirably suited to the sort of synthesis of fundamental knowledge that this situation demands. In auguring this service *Biological Abstracts* will be fulfilling one of the functions for which it was originally intended: that of providing an effective tool for research workers by coordinating the literature of border-line fields.

Under the sectional publication plan this material will be found, at present, not only in Section A, *Abstracts of general biology*, but also under Section B, *Abstracts of experimental animal biology*, Section D, *Abstracts of plant sciences*, and Section E, *Abstracts of animal sciences*.

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE

*A bi-monthly Scientific Journal of Agriculture and the Allied Sciences,
mainly devoted to the publication of the results of original
research and field experiments*

EDITORIAL COMMITTEE

P. M. KHAREGAT, C.I.E., I.C.S., *Vice-Chairman, Imperial Council of
Agricultural Research*

W. BURNS, C.I.E., D.Sc., I.A.S., *Agricultural Commissioner with the Govern-
ment of India*

F. WARE, C.I.E., F.R.C.V.S., F.N.I., I.V.S., *Animal Husbandry Commis-
sioner with the Government of India*

RAO BAHADUR B. VISWANATH, F.I.C., F.C.S., *Director, Imperial Agricultural
Research Institute*

F. C. MINETT, D.Sc., M.R.C.V.S., *Director, Imperial Veterinary Research Institute,
Mukteswar*

J. C. GHOSH, D.Sc., *Director, Indian Institute of Science, Bangalore*

JAMES N. WARNER, M.Sc., *Offg. Professor of Animal Husbandry and Dairying,
Allahabad Agricultural Institute, Allahabad*

S. BASU, I.C.S., *Secretary, Imperial Council of Agricultural Research*

EDITOR

F. M. DE MELLO, B.A., B.Sc. (Econ.)

The Editorial Committee, in the work of examining papers submitted for publication, is assisted in an honorary capacity by a large number of scientists working in various parts of India.

1. Contributions and books and periodicals for review should be addressed to the Editor, Imperial Council of Agricultural Research, Publication Section, New Delhi.

2. All communications regarding subscription and advertisements should be addressed to the Manager of Publications, Civil Lines, Delhi.

CONTENTS

VOL. X, PART II

(April, 1940)

The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal

Original articles—

	PAGE
FINAL REPORT ON THE SCHEME OF INVESTIGATION ON THE WHITE-FLY OF COTTON IN THE PUNJAB	M. Afzal Husain, M.A. (Cantab.) I.A.S. and K. N. Trehan. 101
A NEW PEST (<i>ACANTHIOPHILUS HELIANTHI</i> ROSSI, TRYPETIDAE) OF SAFFLOWER INDIA (WITH PLATE III)	Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.), F. N. I. and H. L. Bhatia, M.Sc. 110
MICRO-BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS, I. CHANGES IN THE CONSTITUENTS OF RICE STRAW (KANAK-TARA) PRODUCED BY MICRO-ORGANISMS PRESENT IN SOIL SUSPENSION UNDER AEROBIC, ANAEROBIC AND WATERLOGGED CONDITIONS (WITH ONE TEXT-FIGURE)	S. S. Guha Sircar ; S. C. De and H. D. Bhowmick 119
II. A NOTE ON THE CHANGES IN THE METHOXYL AND NITROGEN CONTENT OF LIGNIN OF RICE STRAW DURING ITS DECOMPOSITION BY MICRO-ORGANISMS	S. S. Guha Sircar, S. C. De and H. D. Bhowmick 152
STUDIES ON THE CHEMICAL CONSTITUENTS OF INDIAN LATERITIC AND RED SOILS, I. DETERMINATION OF FREE SESQUI-OXIDE COMPONENTS	S. P. Raychaudhuri and M. Sulaiman 158
THE DEPTH OF THE SURFACE LAYER OF THE SOIL TAKING PART IN THE DIURNAL EXCHANGE OF MOISTURE WITH THE AIR LAYERS NEAR THE GROUND	A. K. Mallik, M. Sc., B. Sc. (Ag.), Assoc. I. A. R. I. 164
CONVERSION OF CANE MOLASSES INTO MANURE BY THE BIOLOGICAL METHOD AND THE RESULTS OF THE CROPPING TESTS WITH THE MANURES PREPARED	H. D. Sen, M.Sc., Ph.D. (Lond.), D.I.C. 172
A REVIEW OF THE APPLICATION OF STATISTICAL THEORY TO AGRICULTURAL FIELD EXPERIMENTS IN INDIA	P. C. Mahalanobis. 192
A NOTE ON THE ANALYSIS OF 3 ² AND 3 ⁴ DESIGNS (WITH THREE-FACTOR INTERACTIONS CONFOUNDED) IN FIELD EXPERIMENTS IN AGRICULTURE	M. Vaidyanathan, M.A., L.T., F.S.S. and S. Subramonia Iyer, M.A., F.S.S. 213
Notes	237
Review—	
BIOLOGICAL ABSTRACTS	240